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CLAIMS

[Claim(s)]

[Claim 1] The excess ovulation animal which are the transgenic nonhuman animal which generated to the individual the totipotency cell which introduced the DNA fragment including a promotor array and the DNA array of the array numbers 1 or 2, and its descendant animal, and is characterized by holding the above-mentioned DNA fragment in a somatic cell chromosome.

[Claim 2] The excess ovulation animal of claim 1 whose promotor array is a promotor array of the receptor gene of a gonadotropic hormone.

[Claim 3] The excess ovulation approach characterized by making the protein which is the approach of promoting artificially ovulation of an excess ovulation animal according to claim 1, medicates an animal with the imprint controlling factor of a promotor array, and has the amino acid sequence of the array numbers 3 or 4 discover.

[Claim 4] The excess ovulation approach characterized by making the protein which is the approach of promoting artificially ovulation of an excess ovulation animal according to claim 2, medicates an animal with a gonadotropic hormone, and has the amino acid sequence of the array numbers 3 or 4 discover.

[Claim 5] The excess ovulation animal introduced into the ovary in the oocyte which carried out the transformation by the recombination vector containing a DNA fragment including a promotor array and the DNA array of the array

numbers 1 or 2.
[Claim 6] The excess ovulation animal of claim 5 whose promotor array is a promotor array of the receptor gene of a gonadotropic hormone.
[Claim 7] The excess ovulation approach characterized by making the protein which is the approach of promoting artificially ovulation of an excess ovulation animal according to claim 5, medicates an animal with the imprint controlling factor of a promotor array, and has the amino acid sequence of the array numbers 3 or 4 discover.
[Claim 8] The excess ovulation approach characterized by making the protein which is the approach of promoting artificially ovulation of an excess ovulation animal according to claim 6, medicates an animal with a gonadotropic hormone, and has the amino acid sequence of the array numbers 3 or 4 discover.
[Claim 9] The excess ovulation approach which is an approach of promoting ovulation of a naive animal individual artificially, and is characterized by making the protein which has the amino acid sequence of the array numbers 3 or 4 by medicating an animal individual with protein kinase repressor, SUTAUROSUPORIN, or those derivatives discover.
[Claim 10] The recombination vector containing a DNA fragment including a promotor array and the DNA array of the array number 1.
[Claim 11] The recombination vector containing a DNA fragment including the promotor array of the receptor gene of a gonadotropic hormone, and the DNA array of the array number 1.
[Claim 12] The recombination vector containing a DNA fragment including a promotor array and the DNA array of the array number 2.
[Claim 13] The recombination vector containing a DNA fragment including the promotor array of the receptor gene of a gonadotropic hormone, and the DNA array of the array number 2.
[Claim 14] The cell isolated from the excess ovulation animal according to claim 1 or 2.
[Claim 15] The cell of claim 14 whose cell is a reproductive cell.
[Claim 16] Oocyte which carried out the transformation by the recombination vector containing a DNA fragment including a promotor array and the DNA array of the array numbers 1 or 2.
[Claim 17] Oocyte of claim 16 whose promotor array is a promotor array of the receptor gene of a gonadotropic hormone.

[redacted]

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention relates to the approach of controlling artificially the number of ovulation of the transgenics excess ovulation animal to which the number of ovulation of a mature ovum can be made to increase, and a this excess ovulation animal or a naive animal.

[0002]

[Description of the Prior Art] Many primordial follicles (primodialfollicles) are formed in fetus or after the birth in the ovary of the female individual of mammalian. This primordial follicle is what consists of oocyte which is a reproductive cell which will become an ovum in the future, and a granulosa cell which surround it. The number decided for every sexual cycle through the whole life of an individual these primordial follicles The primary ovarian follicle (primary follicles), The secondary follicle (secondary follicles) and the vesicular ovarian follicle (antral follicles or vesicularfollicles), And it grows to the Graafian follicle (graafian follicles), finally oocyte matures, and it is ovulation (ovulation). The process in which it results is stepped on. However, it is 99.9% although it is only that very few primordial follicles reach an ovulation process as the number of the primordial follicles at the time of birth is restricted by animal species and shown in drawing 1 , and a part finishes with hibernation. A primordial follicle is the "atresia

folliculi" (atresia) at the growth way. It backs through the process said.

[0003] Although age, a breeding cycle, pregnancy, lactation, the hormone balance of ovary inside and outside, a nutrition, ischemia, etc. were mentioned as a factor whose ovarian follicle backs, the detailed molecule mechanism had been unknown for a long time. in recent years, it sees in the case of the atresia folliculi -- from observation of morphological and biochemical and histological change, concentration of nucleus chromatin and nuclear fragmentation are accepted in the granulosa cell of an atretic follicle, and closing of ovarian follicle and the relation of apotosis are suggested. from bacterial research, a gonadotropic hormone controls the apotosis in the atretic follicle of a rat (Tilly et al. and "reference name --) A magazine name, vol.page 1992; Chun" et al. and Endocrinology 135 : 1845-1853 and 1994; Tilly et al. and Endocrinology 136: 1394-14023, 1995; Tilly and Tilly and Endocrinology 136: 242-252 and 1995, A part of the depressant action moreover, the growth hormone in ovarian follicle It minds (). [Tilly et al.,] [Mol.] Endocrinol.6 : 1942-1950, 1992;Chun et al. and Endocrinology 135: 1845-1853, 1994; Tilly et al. and Endocrinology 136: 1394-14023 and 1995 are clear. furthermore, as backing of possibility that apotosis serves as an important point of the optional feature of ovarian follicle The active oxygen in the granulosa cell depending on a gonadotropic hormone (Tilly and Tilly, Endocrinology 136:242-252, 1995), The antioncogene (Tilly et al., Endocrinology 136:1394-14023) of p53 grade, ced-3/interleukin 1 converting enzyme (ICE:) interleukin-1 beta converting enzyme Related gene (Flaws et al., Endocrinology 136:5042-5053) Change is reported.

[0004] However, the detailed elucidation was not made about what kind of function is achieved in the device which controls survival and selection of the ovarian follicle in the ovary containing the oocyte these factors of whose are reproductive cells, or whether the factor which controls apotosis further is related to how [those]. however -- although it was reported very much recently that the Bcl-2 related gene product which controls apotosis and has the prolongation-of-life function of a cell is discovered by the ovarian follicle in the rat ovary (Tilly et al., Endocrinology 136:232-241, 1995) From observation of the mouse which made Bcl-2 gene suffer a loss by gene targetting, it was suggested that Bcl-2 are participating only in the survivability of a primordial follicle (3668 Ratts et al., Endocrinology 136:3665- 1995). Possibility that apotosis repressor different from a Bcl-2 related gene product is involving from this in process of degradation and closing of the primary ovarian follicle which grew from the primordial follicle, the secondary follicle, and the ovarian follicle in the ovary in the vesicular ovarian follicle is assumed.

[0005] On the other hand, in connection with development of the molecule biological technique in recent years, it is

nerve cell apotosis control protein (nural apoptotic inhibitory protein:NAIP) as a gene of cause of the spine nature muscular-atrophy syndrome (spinalmuscular atropy: SMA) which is a familial hereditary disease by positional cloning which is - ** of the technique. It was isolated (Royet al., Cell 80:167-178, 1995). Furthermore, this NAIP gene was introduced into various cultured cells, and when **** to which induction of appointment **-SHISU is carried out was given to the cell, it became clear that that cell death is controlled (Liston et al., Nature 379:349-353, 1996). From these results, possibility that NAIP was a factor which has a cell prolongation-of-life-function in the controlling mechanism of the apotosis which cannot be explained only by the intervention of a Bcl-2 related gene product was suggested.

[0006] In in the living body, the burying-him-alive-cell death called apotosis is a phenomenon indispensable as a device which eliminates an unnecessary cell when maintaining a living body's homeostasis which consists of countless cells. The manifestation of NAIP in an animal individual controls degradation and closing of ovarian follicle, and it not only controls the apotosis of a nerve cell, but is considered to function as keeping constant the number of ovulation programmed by the animal species. Therefore, if it becomes possible to control this NAIP gene expression artificially, the number of ovulation will become possible [producing efficiently the useful animals (livestock animals, such as a cow and a horse etc.) which are fractions] in spite of the ovulation inducing drug processings including the sterility therapy in Homo sapiens.

[0007] This invention is made in view of the situation as above, and aims at offering the excess ovulation animal which holds all the cDNA arrays of a foreignness NAIP gene. Moreover, this invention aims at offering the approach of promoting the number of ovulation of the above-mentioned animal artificially.

[0008] Furthermore, this invention is required for the purpose of offering the approach to which the number of ovulation of a naive animal individual (animal individual into which the foreignness gene is not introduced) including Homo sapiens is made to increase artificially.

[0009]

[Means for Solving the Problem] This invention is the transgenic nonhuman animal which generated to the individual the totipotency cell which introduced the DNA fragment including a promotor array and the DNA array of the array numbers 1 or 2 as what solves the above-mentioned technical problem, and its descendant animal, and offers the excess ovulation animal (claim 1) characterized by holding the above-mentioned DNA fragment in a somatic cell

chromosome.

[0010] Moreover, this invention offers the excess ovulation animal (claim 5) introduced into the ovary in the oocyte which carried out the transformation by the recombination vector containing a DNA fragment including a promotor array and the DNA array of the array numbers 1 or 2. In addition, in these excess ovulation animals, the above-mentioned promotor array makes it the desirable mode to be the promotor array of the receptor gene of a gonadotropic hormone (claims 2 and 6).

[0011] Furthermore, this invention is the approach of promoting artificially ovulation of the above-mentioned excess ovulation animal (claims 1 and 5), medicates an animal with the imprint controlling factor of a promotor array, and offers the excess ovulation approach (claims 3 and 7) characterized by making the protein which has the amino acid sequence of the array numbers 3 or 4 discover. A promotor array is the approach of promoting artificially ovulation of the excess ovulation animal (claims 2 and 6) which is the promotor array of the receptor gene of a gonadotropic hormone, and this invention medicates an animal with a gonadotropic hormone, and offers the excess ovulation approach (claims 4 and 8) characterized by making the protein which has the amino acid sequence of the array numbers 3 or 4 discover further again.

[0012] Furthermore, this invention is the approach of promoting ovulation of a naive animal individual artificially, and offers the excess ovulation approach (claim 9) characterized by making the protein which has the amino acid sequence of the array numbers 3 or 4 discover by medicating an animal individual with protein kinase repressor, SUTAUROSUPORIN, or those derivatives.

[0013] The recombination vector containing the DNA fragment with which this invention besides the above invention includes a promotor array and the DNA array of the array numbers 1 or 2 (claims 10 and 12), The recombination vector containing a DNA fragment including the promotor array of the receptor gene of a gonadotropic hormone, and the DNA array of the array numbers 1 or 2 (claims 11 and 13), The cell (claim 14) isolated from the above-mentioned transgenic nonhuman animal and the oocyte (claim 16) which carried out the transformation by the recombination vector containing a DNA fragment including a promotor array and the DNA array of the array numbers 1 or 2 are offered, respectively.

[0014] Hereafter, the gestalt of implementation of this invention is explained in detail.

[0015]

[Embodiment of the Invention] In this invention, the gene used as a means of degradation / closing control of ovarian follicle is a NAIP gene isolated as a gene of cause of SMA from 5th chromosome macrobrachia of Homo sapiens 13.1 field (5q13.1), and that overall length cDNA has the base sequence of the array number 1 or the array number 2. such cDNA -- for example, 1 section array of the array numbers 1 or 2 -- a probe -- carrying out -- from the cDNA library of the various existing animal origins -- it can isolate -- or a part of array numbers 1 or 2 -- by making an array into a primer, PCR magnification can be carried out and it can obtain. Moreover, the array number 1 or the overall length of the DNA array of two is sufficient as cDNA introduced into an animal individual, or the coding region part is sufficient as it. And in creation of the excess ovulation animal of this invention, this cDNA array and the DNA fragment which connected the promotor array with that upstream are prepared, and the transgenic animal which generated to the individual the totipotency cell which introduced this DNA fragment is created.

[0016] Since a promotor array makes this cDNA array discover within the ovary, it is desirable to use the promotor array of the gene which uses as an imprint controlling factor the matter which exists in an ovary unique target, and the promotor array of the receptor gene of gonadotropic hormones, such as follicle-stimulating hormone and corpus luteal hormone, is desirable especially. Or the promotor array of the foreign gene which uses the matter which is not inherent in the animal species to be used as an imprint controlling factor can also be used. In this case, it can be made discovered, only when introduced cDNA is not spontaneously discovered and prescribes that imprint controlling factor for the patient from the exterior.

[0017] For example, the transgenic animal of this invention can be created by the following approaches. Each reading frame is made in agreement, a promotor array and the DNA array (or that part array) of the array numbers 1 or 2 are ****(ed), a DNA fragment is prepared, and this DNA fragment is introduced into the totipotency cell of a nonhuman mammal. The target animals are useful livestock, such as a cow, Buta, a horse, and a sheep, or are pets, such as a dog, and a cat, the primates, an animal for an experiment, etc. Moreover, as a totipotency cell, a cultured cell like an embryonic stem cell besides a fertilized egg or an early embryo can be used. Impregnation of the DNA fragment to these totipotency cells can use a well-known approach, i.e., an electrostatic pulse method, the liposome method, a calcium phosphate method, a microinjection method, etc. Next, transplant to the oviduct of assumed parents the cell which poured in the DNA fragment, the animal generated to the individual is made born, and it breeds. And a somatic cell is taken out and existence of the DNA fragment which carried out Southern blot analysis of the DNA in this cell,

and introduced it is checked. He is the founder about the individual by which inclusion for the chromosome of a DNA fragment was checked. (Founder) Then, this introductory DNA fragment is transmitted to 50% of that descendant, and can produce the excess ovulation animal of this invention continuously.

[0018] And the excess ovulation animal which carried out in this way and was created has the introductory DNA fragment for the chromosome of all the cells, and makes a promotor array discover cDNA of a NAIP gene by existence of a specific imprint controlling factor. When a promotor array is the promotor of the receptor gene of follicle-stimulating hormone, it is specifically discovered only by the granulosa cell of the ovary in which the hormone exists, and cDNA controls degradation and closing of ovarian follicle. Of course, although the NAIP gene of internality exists in an animal individual and the number of ovulation is controlled, since the excess ovulation animal of this invention discovers degradation / closing repressor of ovarian follicle so much with the NAIP gene cDNA of the introduced foreignness, compared with a naive animal, a lot of ovulation is possible for it. Moreover, excess ovulation is also artificially controllable by medicating a promotor with a specific imprint controlling factor.

[0019] On the other hand, the excess ovulation animal of this invention can be created also by introducing the above-mentioned DNA fragment directly in the ovary of an animal individual. That is, the recombination vectors (an adenovirus vector, retrovirus vector, etc.) incorporating a DNA fragment are transplanted to the ovary of an animal individual by the physical approach. Or the oocyte which carried out the transformation by the above-mentioned recombination vector is transplanted to the ovary. A NAIP gene carries out the abundant manifestation also of such an animal rather than a naive animal into the ovary, and it serves as excess ovulation. Moreover, it is possible to control the excess ovulation by prescribing for the patient the imprint controlling factor of the promotor array connected with the NAIP gene cDNA.

[0020] This invention also offers the excess ovulation approach of promoting ovulation of a naive animal individual artificially further again. That is, all animal individuals are equipped with the NAIP gene into that genome, and the gene of this internality can be made to discover transient by carrying out whole body administration of protein kinase repressor (for example, immunosuppresant FK506 grade), or SUTAUROSUPORIN (K252A etc.) or these derivatives. A lot of ovulation is attained in an animal individual including Homo sapiens, without using an ovulation inducing drug etc., and insurance and a positive sterility therapy are offered by this.

[0021] Next, the experimental result which checked that a NAIP gene was a gene which surely participates in

degradation / closing control of ovarian follicle is shown, and the effectiveness of this invention is explained.
(1) Humidity and temperature were bred under the environment adjusted uniformly, and superovulation processing of an ingredient, an approach animal, and the gonadotropic hormone processing ICR system female mouse (it purchases from Japanese Clare, Inc.) was carried out for ** term 12 hours (5:00 - 17:00) at the time of 3 weeks old. That is, the pregnant-mare-serum gonadotropic hormone (pregnant mare serum gonadotropin, PMSG) of 5IU was injected intraperitoneally, and the Homo sapiens chorionic gonadotropin (human chorionic gonadotropin, hCG) of 5IU was injected intraperitoneally similarly 48 hours after.
in Carry out paraffin embedding after carrying out dehydration **** of the mouse ovary which carried out situ hybridization BUAN immobilization, and it is 5-6 micrometers in thickness. They are 10 ** to the slide glass which carried out silane coat **** after thin sectioning at the intercept. After making it dry, the organization intercept which performed deparaffinization row hydrophilic actuation according to the conventional method was ****(ed) 0.3% by Protease (Triton-X (2 between parts), 0.2 %HCl (for 20 minutes), and 20microg/ml) (for 20 minutes) K. Furthermore, after being immersed in the fresh paraformaldehyde of 4 % for 5 minutes and making it re-fix to it, it processed in the 0.2 % glycine for 1 hour, residual ARUDEHITO was neutralized, and it ****(ed) by formamide 50% as pre-hybridization for 2 hours.
[0022] In antisense one of NAIP and the sense RNA probe which are used for high buri die ZEJON Homo sapiens naip gene (Roy et al. --) Cell 80:167-178 and 195 of 1995 A part of BIR1, BIR2, and BIR3 of the BIR (baculoviral inhibition of apoptosis protein repeat) field from a base to 1263 bases It is a subclo to pBluscript about the included gene fragment (drawing 2). - The vector which carried out NINGU is used. Digoxigenin (Digoxigenin:DIG)-RNA labeling Kit of Boehringer Mannheim The generated DIG indicator RNA probe was used by using and carrying out an in vitro imprint. In addition, about this PU opening 1 BU, the gay opening G with mouse c-IAP1 which is an IAP (inhibitor of apoptosis protein) related gene is 30 - 40%, and is understood that crossover nature is low. Hybridization is 50% formamide, 10% dextran sulfate, 1X Denhardt's solution, and 100. It carried out to ****** of mug/ml single strand salmon sperm DNA, 100microg [/ml] Escherichia coli tRNA, and 10 mM DICHIOSUTE oar under the 50-degree C condition in the moisture chamber for 16 to 20 hours using 65 degrees C and the solution which added the RNA probe denatured by the processing for 5 minutes beforehand. Next, in order to remove an unreacted RNA probe from the preparation, the following washing actuation was performed. First, after washing for 20 minutes in 4XSSC, processing

for 30 minutes and washing 37 degrees C in 20microg [/ml] RNase, it reaches 2 XSSC under a 68 more-degree C condition. It washed in 0.2XSSC for 1 hour each.

[0023] 5-bromo-4-chloro-3-indoyl-phosphate-nitroblue tetrazolium after making the alkali FOSUFATA 1 ZE indicator DIG antibody of Boehringer Mannheim react in detection by the immunohistochemistry reaction (BCIP/NBT) The signal in which existence of RNA of a NAIP gene is shown according to considering as a substrate and making it color under 4-degree C conditions was detected. About creation of BUAN immobilization of the histochemistry-detection mouse ovary of apotosis, paraffin embedding, and an organization intercept, it applied to the above-mentioned approach correspondingly. Terminal Deoxynucleotidly Transferase-(TdT) mediated dUTP-biotin nick end labeling (TUNEL) whose detection of morphological apotosis is the approach of detecting fragmentation in the NUKURESOMU unit of Chromosome DNA in histochemistry -- law was used. After using MEBSTAIN Kit (Medical & Biological Laboratories) fundamentally, carrying out PU opening TEAZE processing of the organization intercept first and making the amount of [of the fragmentation DNA in a nucleus] 3'OH end incorporate biotin-ized dUTP, add streptoavidin-HRP (horseradish peroxidase), combined TUNEL assay, it was made to color using DAB (diaminobenzidine) as a substrate, and Fragmentation DNA was detected to it.

The ovary is extracted from the ICR juvenile female mouse of isolation of a granulosa cell, and 3 weeks old culture, fat tissue, blood, etc. are removed on a filter paper, and it is an Eagle's minimum essential medium (minimum essential medium:MEM). It offered as a sample to the experiment after washing. the isolation and culture of a cell lump containing oocyte of a granulosa cell -- Eppig ** -- (Biol.Reprod., 41:268-276, 1989) Hirano and others (J.Exp.Zool., 267:543-547, 1993) It carried out almost according to the reported approach. First, it was immersed and the ovary extracted to the MEM which added 2mg [/ml] collagenase (the object for cell distribution, Wako Pure Chem) was processed for 30 minutes. Pipetting was repeated with Pasteur pipette after washing, and the cell lump of the granulocyte containing oocyte was isolated. 4mg [/ml] bovine serum albumin in the culture plate in which the cell lump which isolated did the coat with the agar -- and -- It cultivated by 2mM hypoxanthine. Ten days after culture initiation, the cell lump of the granulosa cell containing these oocytes was moved to the MEM containing 100 ng(s)/ml follicle-stimulating hormone (fillicle-stimulating hormone: FSH and sigma), the cell lump of the granulosa cell which contains oocyte 18 hours after was extracted, and the analysis of NAIP gene expression was presented.

an RNA extract and the extract of all RNA from reverse transcriptase PCR (RT-PCR) and the northern-blot-analysis

ovary -- AGPC (acid guanidium thiocyanate-phenol-chloroform) -- law (Anal.Biochem.162:156-159, 1987) It carried out. After all extracted RNA digested the genomic DNA mixed by carrying out DNaseI processing, the quantum of it was carried out and the experiment was presented with it.

[0024] By RT-PCR analysis, it is 1microg. From all RNA to AMVXL Therefore, cDNA was compounded to RTase (TAKARA SHUZO), and cDNA of Mouse naip was amplified using the part. the primer used for magnification -- 5 -- '-CACAGGGGTGAAACTTGGGGTTCAG-3' 5 [and] -- '-CACCTGTGGTTTCCATGGCTTCTGG-3' it is -- after the reaction condition carried out 94 degrees C of thermal denaturation for 5 minutes, it wound annealing 60 degree C for 94 degrees C of thermal denaturation for 1 minute, wound the cycle for 2 minutes for 1 minute and for 72 degrees C of DNA synthesis 40 times, and removed. Electrophoresis of the amplified RT-PCR product was carried out by agarose gel 2%, and it was separated and detected.

[0025] 6-8microg refined in NO 1 Zhang blotting analysis using Oligotex-dT<Super> (TAKARA SHUZO) from all RNA (Pori A) +RNA was used. It is a nylon filter after separating agarose electrophoresis under existence of a formamide. It transferred to ybondN+ (Amersham). UV irradiation was carried out to the dried nylon filter on the next day, and RNA on a filter was fixed. ExressHyb hybridization solution (Clonetec) was used in pre hybridization **** hybridization. First, the mouse nnaipcDNA probe which carried out 32 P-dCTP indicators was added after the pre hybridization of 2 - 3 hours, and hybridization was performed overnight. In addition, the gene fragment used for the probe is 473 of a mouse naip gene (Robertson et al., unpublished data). 854 containing a part of BIR1 from a base to 1326 bases, and BIR2 and BIR3 It is the gene fragment of the die length of a base (drawing 2). Too, the homology with mouse c-IAP1 which is an IAP related gene is 30 - 40%, and is understood that crossover nature is low. Washing of hybridization is [a room temperature and] for 20 minutes in the water solution of 0.1XSSC and 0.1 %DSD to 2 times and a degree in the water solution of 2XSSC and 0.05%SDS 68 degrees C and 20 Between parts was performed by carrying out twice. The washed filter performed autoradiography by the imaging plate (FUJI), detected the signal by BAS-2000 and performed quantitative analysis.

(2) in situ hybridization using a specific RIBOPU lobe [as opposed to the cDNA for the NAIP gene expression in the ovary extracted from the female mouse of the weeks old of NAIP gene expression versatility accompanying growth of the ovarian follicle in the result ovary] By investigating, the NAIP gene expression accompanying growth of the ovarian follicle in the ovary was examined.

[0026] Although the ovarian follicle images in the ovary observed in 2 age-in-day mouse ovary immediately after birth were two ovarian follicle images of the primordial follicle by which oocyte was surrounded by much more flat granulosa cell or the granulosa cell which became cube-like, oocyte, and the primary ovarian follicle which consists of basement membrane of a periphery, in a primordial follicle, a difference was not regarded as the case where sense and an antisense RIBOPU lobe are made to hybridize, either. The strong signal was observed by the time of using an antisense RIBOPU lobe in the granulosa cell of the primary ovarian follicle which has the oocyte which grew more on the other hand (drawing 3 (A)). Next, when NAIP gene expression was investigated in the mouse ovary in which 12 weeks old which repeats sexual cycle regularly matured, NAIP gene expression was accepted also in the cumulus cell which surround the granulosa cell and oocyte of a Graafian follicle which are vesicular ovarian follicle in front of ovulation (drawing 3 (B)). [0027] From the above result, having discovered the NAIP gene by the granulosa cell from the primary ovarian follicle to the Graafian follicle in front of ovulation was checked.

NO 1 Zhang blotting analysis investigated the NAIP gene expression in each organization of the mouse containing the tissue-specific-expression ovary of a NAIP gene by using Mouse naipcDNA as a probe.

[0028] The NAIP gene is discovered as a transcript with two die length in mouse each organization including the ovary. First, 2microg Pori Although the strong signal was seen with ****, lungs, liver, and the heart when the NAIP gene expression in the main organizations of a mouse was examined using mouse multiple-tissue northernblot (MTN blot, Clonetec) which combined (A)+RNA, there is no tissue specificity and the manifestation was mostly accepted in all organizations (drawing 4 (A)). On the other hand about a manifestation in the ovary, 2 age in day, 3 weeks old, 12 weeks old, and the 3rd day of a delivery And 8microg of the ovary origin extracted from the various female mice of 18 weeks old Pori The place which used the filter which carried out BUROTSU ** of the (A)+RNA, Strong NAIP gene expression was observed with the female mouse of 12 weeks old and 18 weeks old which repeats sexual cycle with many rates of primary, the secondary follicle, or the vesicular ovarian follicle and which matured. However, in the ovary of the 2 age in day and the day [of a delivery / 3rd] female mouse with which a primordial follicle and a corpus luteum occupy many, respectively, NAIP gene expression was weak.

[0029] From the above thing, it was checked that the NAIP gene expression in the ovary is in the development process and correlation of ovarian follicle.

The localization gonadotropic hormone of the NAIP gene expression in ovarian follicle investigated the manifestation

of a NAIP gene with time in the ovary of the female mouse of 3 weeks old which gave superovulation ****. the amount of NAIP gene expression in the ovary 48 hours [with the operation as follicle-stimulating hormone (FSH)] after PMSG administration -- before administration -- comparing -- about 1.6 It is twice and has a corpus luteal hormone (Lutinating hormone, LH) operation further. administration hours [7 hours] after hCG -- administration before -- comparing -- about 2.4 twice -- the strong manifestation was observed. When RT-PCR detected the NAIP gene expression in the cell lump of the granulosa cell which contains next the oocyte isolated from the ovary, it was shown by oocyte that the manifestation is discovered only by private seal ** and the granulosa cell. Furthermore, although there was no absolute quantum nature for magnification by PCR, the inclination for a NAIP gene expression signal to become strong with a gonadotropic hormone was observed.

[0030] It was checked that carry out localization of the NAIP gene expression to the granulosa cell of ovarian follicle, and the manifestation is reinforced with gonadotropic hormones, such as FSH, from the above result.

It sets on the continuation organization intercept of the related ovary of the atresia folliculi and NAIP gene expression, and is in situ hybridization. Comparison examination of the apotosis accepted by the NAIP gene expression detected and the TUNEL assay was carried out. Consequently, as shown in drawing 5 , apotosis was not observed in the granulosa cell of the ovarian follicle which the NAIP gene has discovered strongly. On the other hand, by the atretic follicle characterized by deformation of oocyte, the granulosa cell which carried out cell death was observed, and by such ovarian follicle, NAIP gene expression is feeble or was hardly observed. [0031] Since the NAIP gene was not discovered by the closed ovarian follicle as above, it was checked that the NAIP gene is functioning as apotosis repressor in the ovarian follicle in the ovary.

[0032]

[Effect of the Invention] The approach of promoting ovulation artificially is offered by controlling NAIP gene expression by this invention with the excess ovulation animal which introduced the NAIP gene which controls degradation and closing of ovarian follicle as explained in detail above. By this, development of a new sterility therapy can be attained and the productivity of a useful animal can also be raised.

[0033]

[Layout Table]

array number: -- die-length [of one array]: -- mold [of 5984 arrays]: -- number [of nucleic-acid chains]: --

double strand topology: -- class [of straight chain-like array]: -- cDNA to mRNA origin living thing name: -- Homo sapiens array ACAAAAGGTC CTGTGCTCAC CTGGGACCCT TCTGGACGTT GCCCTGTGTT CCTCTTCGCC 60
TGCCTGTTCA TCTACGACGA ACCCCGGGTA TTGACCCCAG ACAACAATGC CACTTCATAT 120 TGGGGACTTC
GTCTGGGATT CCAAGGTGCA TTCATTGCAA AGTTCCTTAA ATATTTTCTC 180 ACTGCTTCCT ACTAAAGGAC
GGACAGAGCA TTTGTTCTTC AGCCACATAC TTTCCTTCCA 240 CTGGCCAGCA TTCTCCTCTA TTAGACTAGA
ACTGTGGATA AACCTCAGAA AATGGCCACC 300 CAGCAGAAAG CCTCTGACGA GAGGATCTCC CAGTTTGATC
ACAATTTGCT GCCAGAGCTG 360 TCTGCTCTTC TGGGCCTAGA TGCAGTTCAG TTGGCAAAGG AACTAGAAGA
AGAGGAGCAG 420 AAGGAGCGAG CAAAAATGCA GAAAGGCTAC AACTCTCAAA TGCGCAGTGA AGCAAAAAGG
480 TTAAAGACTT TTGTGACTTA TGAGCCGTAC AGCTCATGGA TACCACAGGA GATGGCGGCC 540
GCTGGGTTTT ACTTCACTGG GGTAAAATCT GGGATTCAGT GCTTCTGCTG TAGCCTAATC 600 CTCTTTGGTG
CCGGCCTCAC GAGACTCCCC ATAGAAGACC ACAAGAGGTT TCATCCAGAT 660 TGTGGGTTCC TTTTGAACAA
GGATGTTGGT AACATTGCCA AGTACGACAT AAGGGTGAAG 720 AATCTGAAGA GCAGGCTGAG AGGAGGTAAA
ATGAGGTACC AAGAAGAGGA GGCTAGACTT 780 GCATCCTTCA GGAACTGGCC ATTTTATGTC CAAGGGATAT
CCCCTTGTGT GCTCTCAGAG 840 GCTGGCTTTG TCTTTACAGG TAAACAGGAC ACGGTACAGT GTTTTTCCTG
TGGTGGATGT 900 TTAGGAAATT GGGAAGAAGG AGATGATCCT TGGAAGGAAC ATGCCAAATG GTTCCCCAAA
960 TGTGAATTTC TTCGGAGTAA GAAATCCTCA GAGGAAATTA CCCAGTATAT TCAAAGCTAC 1020
AAGGGATTTG TTGACATAAC GGGAGAACAT TTTGTGAATT CCTGGGTCCA GAGAGAATTA 1080 CCTATGGCAT
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chains]: -- double strand topology: -- class [of straight chain-like array]: -- cDNA to mRNA origin living thing
name: -- Homo sapiens array ACAAAAGGTC CTGTGCTCAC CTGGGACCCT TCTGGACGTT GCCCTGTGTT
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375 380 Ile Val Pro Glu Met Ala Gln Gly Glu Ala Gln Trp Phe Gln Glu Ala 385 390 395 400 Lys Asn Leu Asn Glu Gln Leu Arg Ala Ala Tyr Thr Ser Ala Ser Phe 405 410 415 Arg His Met Ser LeuLeu Asp Ile Ser Ser Asp Leu Ala Thr Asp His 420 425 430 Leu Leu GlyCysAsp Leu Ser Ile Ala Ser Lys His Ile Ser Lys Pro 435 440 445 Val Gln Glu Pro Leu Val Leu Pro Glu Val Phe Gly Asn Leu Asn Ser 450 455 460 Val Met Cys Val Glu Gly Glu Ala Gly Ser Gly Lys Thr Val Leu Leu 465 470 475 480 Lys Lys Ile Ala Phe Leu Trp Ala Ser Gly Cys Cys Pro Leu Leu Asn 485 490 495 Arg Phe Gln Leu Val Phe Tyr Leu Ser Leu Ser Ser Thr Arg Pro Asp 500 505 510 Glu Gly LeuAla SerIle Ile Cys Asp Gln Leu Leu Glu Lys Glu Gly 515 520 525 Ser Val Thr Glu Met Cys Met Arg Asn Ile Ile Gln Gln Leu Lys Asn 530 535 540 Gln Val Leu Phe Leu Leu Asp Asp Tyr Lys Glu Ile Cys Ser Ile Pro 545 55 0 555 560 Gln Val Ile Gly Lys Leu Ile Gln Lys-Asn-His-Leu-Ser Arg Thr Cys 565 570 575 Leu Leu Ile Ala Val Arg Thr Asn Arg-Ala-Arg-Asp-Ile Arg Arg Tyr 580 585590 Leu Glu ThrIle Leu Glu Ile Lys Ala Phe Pro Phe Tyr Asn Thr Val 595 600 605 Cys Ile Leu Arg Lys Leu Phe Ser His Asn Met Thr Arg Leu Arg Lys 610 615 620 Phe Met ValTyr Phe Gly LysAsn Gln Ser Leu Gln Lys Ile Gln Lys 625 630 635 640 Thr Pro Leu Phe Val Ala Ala Ile Cys Ala His Trp Phe Gln Tyr Pro 645 650 655 Phe Asp Pro Ser Phe Asp Asp Val Ala Val Phe Lys Ser Tyr Met Glu 660 665 670 Arg Leu SerLeuArg Asn Lys Ala Thr Ala Glu Ile Leu Lys Ala Thr 675 680 685 Val Ser Ser Cys Gly Glu Leu Ala Leu Lys Gly Phe Phe Ser Cys Cys 690 695 700 Phe Glu Phe Asn Asp Asp Asp Leu Ala Glu Ala Gly Val Asp Glu Asp 705 710 715 720 Glu Asp Leu Thr Met Cys Leu Met Ser Lys Phe Thr Ala Gln Arg Leu 725 730 735 Arg Pro Phe Tyr Arg Phe Leu Ser Pro Ala Phe Gln Glu Phe Leu Ala 740 745 750 Gly Met ArgLeu Ile Glu Leu Leu Asp Ser Asp Arg Gln Glu His Gln 755 760 765 Asp Leu Gly Leu Tyr His Leu Lys Gln Ile Asn Ser Pro Met Met Thr 770 775 780 Val Ser Ala Tyr Asn Asn Phe Leu Asn Tyr Val Ser Ser Leu Pro Ser 785 790 795 800 Thr Lys Ala Gly Pro Lys Ile Val Ser His Leu Leu His Leu Val Asp 805 810 815 Asn Lys Glu Ser Leu Glu Asn Ile Ser Glu Asn Asp Asp Tyr Leu Lys 820 825 830 His Gln Pro Glu Ile Ser Leu Gln Met Gln Leu Leu Arg Gly Leu Trp 835 840 845 Gln Ile CysProGln Ala Tyr Phe Ser Met Val Ser Glu His Leu Leu 850 855 860 Val Leu Ala Leu Lys Thr Ala Tyr Gln Ser Asn Thr Val Ala Ala Cys 865 870 875 880 Ser Pro Phe Val Leu Gln Phe Leu Gln-Gly-Arg-Thr-Leu Thr Leu Gly 885 890 895 Ala Leu Asn Leu Gln Tyr Phe Phe Asp-His-Pro-Glu Ser Leu Ser Leu 900905 910 Leu Arg Ser Ile His Phe Pro Ile Arg Gly Asn Lys Thr Ser Pro Arg 915 920 925 Ala His Phe Ser Val Leu Glu Thr Cys Phe Asp Lys Ser Gln Val Pro 930 935 940 Thr Ile Asp Gln Asp Tyr Ala Ser Ala Phe Glu Pro Met Asn Glu Trp 945 950 955 960 Glu Arg Asn Leu Ala Glu Lys Glu Asp Asn Val Lys Ser Tyr Met Asp 965 970 975 Met Gln Arg Arg Ala Ser Pro Asp Leu Ser Thr Gly Tyr Trp Lys Leu 980 985 990 Ser Pro LysGln Tyr Lys Ile Pro Cys Leu Glu Val

Asp Val Asn Asp 995 1000 1005 Ile Asp Val Val Gly Gln Asp Met Leu Glu Ile Leu Met Thr Val Phe 1010 1015 1020 Ser Ala Ser Gln Arg IleGlu Leu His Leu Asn His Ser Arg Gly Phe 1025 1030 1035 1040 Ile Glu Ser Ile Arg Pro Ala Leu Glu Leu Ser Lys Ala Ser Val Thr 1045 1050 1055 Lys Cys Ser Ile Ser Lys Leu Glu Leu Ser Ala Ala Glu Gln Glu Leu 1060 1065 1070 Leu Leu ThrLeu Pro Ser Leu Glu Ser Leu Glu Val Ser Gly Thr Ile 1075 1080 1085 Gln Ser Gln Asp Gln Ile Phe Pro Asn Leu Asp Lys Phe Leu Cys Leu 1090 1095 1100 Lys Glu Leu Ser Val Asp Leu Glu Gly Asn Ile Asn Val Phe Ser Val 1105 1110 1115 1120 Ile Pro Glu Glu Phe ProAsn Phe His His Met Glu Lys Leu Leu Ile 1125 1130 1135 Gln Ile Ser Ala Glu Tyr Asp Pro Ser Lys Leu Val Lys Leu Ile Gln 1140 11451150 Asn Ser Pro Asn Leu His ValPhe His Leu Lys Cys Asn Phe Phe Ser 1155 1160 1165 Asp Phe Gly Ser-Leu-Met-Thr-Met Leu Val Ser Cys Lys-Lys-Leu-Thr 1170 1175 1180 Glu Ile Lys Phe-Ser-Asp-Ser-Phe Phe Gln Ala Val Pro Phe Val Ala 1185 1190 1195 1200 Ser Leu Pro Asn Phe Ile Ser Leu Lys Ile Leu Asn Leu Glu Gly Gln 1205 1210 1215 Gln Phe Pro Asp Glu Glu Thr Ser Glu Lys Phe Ala Tyr Ile Leu Gly 1220 1225 1230 Ser Leu Ser Asn Leu Glu Glu Leu Ile Leu Pro Thr Gly Asp Gly Ile 1235 12401245 Tyr Arg ValAla Lys Leu Ile Ile Gln Gln Cys Gln Gln Leu His Cys 1250 1255 1260 Leu Arg Val Leu Ser Phe Phe LysThr Leu Asn Asp Asp Ser Val Val 1265 1270 1275 1280 Glu Ile Ala Lys Val Ala Ile Ser Gly Gly Phe Gln Lys Leu Glu Asn 1285 1290 1295 Leu Lys Leu Ser Ile Asn His LysIle Thr Glu Glu Gly Tyr Arg Asn 1300 1305 1310 Phe Phe Gln Ala Leu Asp Asn Met Pro Asn Leu Gln Glu Leu Asp Ile 1315 1320 1325 Ser Arg HisPhe Thr Glu Cys Ile Lys Ala Gln Ala Thr Thr Val Lys 1330 1335 1340 Ser Leu Ser Gln Cys Val Leu Arg Leu Pro Arg Leu Ile Arg Leu Asn 1345 1350 1355 1360 Met Leu Ser Trp Leu Leu Asp Ala Asp Asp Ile Ala Leu Leu Asn Val 1365 1370 1375 Met Lys Glu Arg His Pro Gln Ser Lys Tyr Leu Thr Ile Leu Gln Lys 1380 1385 1390 Trp Ile Leu Pro Phe Ser ProIle Ile Gln Lys 1395 1400 1403 array number: -- die-length [of four arrays]: -- mold [of 1295 arrays]: -- amino acid topology: -- class [of straight chain-like array]: -- protein array Met Ala Thr Gln Gln Lys Ala Ser Asp Glu Arg Ile Ser Gln Phe Asp 1 5 10 15 His Asn Leu Leu Pro Glu Leu Ser Ala Leu Leu Gly Leu Asp Ala Val 20 25 30 Gln Leu Ala Lys Glu Leu Glu Glu Glu Glu Gln Lys Glu Arg Ala Lys 35 40 45 Met Gln LysGly Tyr Asn Ser Gln Met Arg Ser Glu Ala Lys Arg Leu 50 55 60 Lys Thr Phe Val Thr Tyr Glu Pro Tyr Ser Ser Trp-Ile-Pro-Gln-Glu 65 70 75 80 Met Ala Ala Ala-Gly-Phe-Tyr-Phe-Thr Gly Val Lys Ser Gly-Ile-Gln 85 90 95 Cys Phe Cys Cys-Ser-Leu-Ile-Leu-Phe Gly Ala Gly Leu Thr Arg Leu 100 105 110 Pro Ile Glu Asp His Lys Arg Phe His Pro Asp Cys Gly Phe Leu Leu 115 120 125 Asn Lys Asp Val Gly Asn Ile Ala Lys Tyr Asp Ile Arg Val Lys Asn 130 135 140 Leu Lys Ser Arg Leu Arg Gly Gly Lys Met Arg Tyr Gln Glu Glu Glu 145 150 155 160 Ala Arg Leu Ala Ser Phe Arg Asn Trp Pro Phe Tyr Val Gln Gly Ile 165 170

175 Ser Pro Cys Val Leu Ser Glu Ala Gly Phe Val Phe Thr Gly Lys Gln 180 185 190 Asp Thr Val Gln Cys Phe Ser
Cys Gly Gly Cys Leu Gly Asn Trp Glu 195 200 205 Glu Gly Asp Asp Pro Trp Lys Glu His Ala Lys Trp Phe Pro Lys
Cys 210 215 220 Glu Phe Leu Arg Ser Lys Lys Ser Ser Glu Glu Ile Thr Gln Tyr Ile 225 230 235 240 Gln Ser Tyr Lys
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Ala-Ala-Tyr-Thr-Ser Ala Ser Phe 405 410 415 Arg His Met Ser Leu Leu Asp Ile Ser Ser Asp Leu Ala ThrAspHis
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Leu 465 470 475 480 Lys Lys Ile Ala Phe Leu Trp Ala Ser Gly Cys Cys Pro Leu Leu Asn 485 490 495 Arg Phe Gln
Leu Val Phe Tyr LeuSer Leu Ser Ser Thr Arg Pro Asp 500 505 510 Glu Gly LeuAlaSer Ile Ile Cys Asp Gln Leu Leu
Glu Lys Glu Gly 515 520 525 Ser Val Thr Glu Met Cys Met Arg Asn Ile Ile Gln Gln Leu Lys Asn 530 535 540 Gln Val
Leu Phe Leu Leu Asp Asp Tyr Lys Glu Ile Cys Ser Ile Pro 545 550 555 560 Gln Val Ile Gly Lys Leu Ile Gln Lys Asn
His Leu Ser Arg Thr Cys 565 570 575 Leu Leu Ile Ala Val Arg Thr Asn Arg Ala Arg Asp Ile Arg Arg Tyr 580 585 590
Leu Glu Thr Ile LeuGlu Ile Lys Ala Phe Pro Phe Tyr Asn Thr Val 595 600 605 Cys Ile Leu Arg Lys Leu Phe Ser His
Asn Met Thr Arg Leu Arg Lys 610 615 620 Phe Met Val Tyr Phe Gly Lys Asn GlnSer Leu Gln Lys Ile Gln Lys 625 630
635 640 Thr Pro Leu Phe Val Ala Ala Ile Cys Ala His Trp Phe Gln Tyr Pro 645 650 655 Phe Asp Pro Ser Phe Asp Asp
Val Ala Val Phe Lys Ser Tyr Met Glu 660 665 670 Arg Leu Ser LeuArg Asn Lys Ala Thr Ala Glu Ile Leu Lys Ala Thr
675 680 685 Val Ser Ser Cys Gly Glu Leu Ala Leu Lys Gly Phe Phe Ser Cys Cys 690 695 700 Phe Glu Phe Asn Asp
Asp Asp Leu-Ala-Glu-Ala-Gly Val Asp Glu Asp 705 710 715 720 Glu Asp Leu Thr Met Cys Leu Met
Ser-Lys-Phe-Thr-Ala Gln Arg Leu 725 730 735 Arg Pro Phe Tyr-Arg Phe Leu Ser Pro Ala Phe Gln Glu Phe Leu Ala
740745 750 Gly Met Arg Leu Ile Glu Leu Leu Asp Ser Asp Arg Gln Glu His Gln 755 760 765 Asp Leu Gly Leu Tyr His
Leu Lys Gln Ile Asn Ser Pro Met Met Thr 770 775 780 Val Ser Ala Tyr Asn Asn Phe Leu Asn Tyr Val Ser Ser Leu Pro

Ser 785 790 795 800 Thr Lys Ala Gly Pro Lys Ile Val Ser His Leu Leu His Leu Val Asp 805 810 815 Asn Lys Glu Ser Leu Glu Asn Ile Ser Glu Asn Asp Asp Tyr Leu Lys 820 825 830 His Gln ProGlu Ile Ser Leu Gln Met Gln Leu Leu Arg Gly Leu Trp 835 840 845 Gln Ile Cys Pro Gln Ala Tyr Phe Ser Met Val Ser Glu His Leu Leu 850 855 860 Val Leu Ala Leu Lys Thr AlaTyr Gln Ser Asn Thr Val Ala Ala Cys 865 870 875 880 Ser Pro Phe Val Leu Gln Phe Leu Gln Gly Arg Thr Leu Thr Leu Gly 885 890 895 Ala Leu Asn Leu Gln Tyr Phe Phe Asp His Pro Glu Ser Leu Ser Leu 900 905 910 Leu ArgSerIle His Phe Pro Ile Arg Gly Asn Lys Thr Ser Pro Arg 915 920 925 Ala His Phe Ser Val Leu Glu Thr Cys Phe Asp Lys Ser Gln Val Pro 930 935 940 Thr Ile Asp Gln Asp Tyr Ala SerAla Phe Glu Pro Met Asn Glu Trp 945 950 955 960 Glu Arg Asn Leu Ala Glu Lys Glu Asp Asn Val Lys Ser Tyr Met Asp 965 970 975 Met Gln Arg Arg Ala Ser Pro Asp Leu Ser Thr Gly Tyr Trp Lys Leu 980 985 990 Ser Pro Lys Gln Tyr Lys Ile Pro Cys-Leu-Glu-Val-Asp Val Asn Asp 995 1000 1005 Ile Asp Val Val-Gly-Gln-Asp-Met Leu Glu Ile Leu Met-Thr-Val-Phe 1010 1015 1020 Ser Ala Ser Gln Arg Ile Glu Leu His Leu Asn His Ser Arg Gly Phe 1025 1030 1035 1040 Ile Glu Ser Ile Arg Pro Ala Leu Glu Leu Ser Lys Ala Ser Val Thr 1045 1050 1055 Lys Cys Ser Ile Ser Lys Leu Glu Leu Ser Ala Ala Glu Gln Glu Leu 1060 1065 1070 Leu Leu Thr Leu Pro Ser Leu Glu Ser Leu Glu Val Ser Gly Thr Ile 1075 1080 1085 Gln Ser GlnAsp Gln Ile Phe Pro Asn Leu Asp Lys Phe Leu Cys Leu 1090 1095 1100 Lys Glu Leu Ser Val Asp Leu Glu Gly Asn Ile Asn Val Phe Ser Val 1105 1110 1115 1120 Ile Pro Glu Glu Phe Pro Asn Phe His His Met Glu Lys Leu Leu Ile 1125 1130 1135 Gln Ile Ser Ala Glu Tyr Asp ProSer Lys Leu Val Lys Leu Ile Gln 1140 1145 1150 Asn Ser Pro Asn LeuHis Val Phe His Leu Lys Cys Asn Phe Phe Ser 1155 11601165 Asp Phe GlySer Leu Met Thr Met Leu Val Ser Cys Lys Lys Leu Thr 1170 1175 1180 Glu IleLys Phe Ser Asp Ser Phe Phe Gln Ala Val Pro Phe Val Ala 1185 1190 1195 1200 Ser Leu Pro Asn Phe Ile Ser Leu Lys Ile Leu Asn Leu Glu Gly Gln 1205 1210 1215 Gln Phe Pro Asp Glu Glu Thr Ser Glu Lys Phe Ala Tyr Ile Leu Gly 1220 1225 1230 Ser Leu Ser Asn Leu Glu Glu Leu Ile Leu Pro Thr Gly Asp Gly Ile 1235 1240 1245 Tyr Arg Val Ala Lys Leu Ile Ile Gln Gln Cys Gln Gln Leu His Cys 1250 1255 1260 Leu Arg Val Leu Ser Phe Phe Lys ThrLeu Asn Asp Asp Ser Val Val 1265 1270 1275 1280 Glu Ile Gly Glu Leu Val Phe Gln Leu Ala Trp Lys Pro Val Val 1285 1290 1295

[redacted]

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1] It is the mimetic diagram having shown the development process of the ovarian follicle in the ovary.

[Drawing 2] It is the mimetic diagram having shown the gene location of the probe for hybridization.

[Drawing 3] in situ hybridization which investigated the NAIP gene expression in the ovary It is a result and is (A). It is (B) when a sense RIBOPU lobe is used. The case where an antisense RIBOPU lobe is used is shown.

[Drawing 4] It is as a result of Northern blot analysis of a mouse NAIP gene. (A) The gene expression in ** mouse each organization is shown, and each lane is 1:testis, 2:kidney, 3:skeletal muscle, 4:liver, 5:lungs, 6:spleen, 7:brain, and 8:heart. (B) The gene expression within the ovary in the development process of a ** mouse is shown, and each lane is 1:2 age in day, 2:3 weeks old, 3:12 weeks old, and the 3rd day of 4:delivery and 5:18 weeks old.

[Drawing 5] It is as a result of in situ hybridization which showed that the NAIP gene expression in the ovary was carrying out localization to the granulosa cell, and (an upper case, interruption) a TUNEL assay (lower berth).

[Translation done.]

DRAWINGS

[Drawing 1]

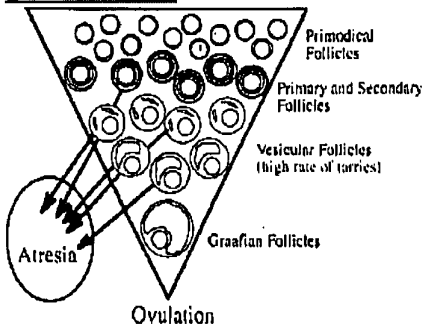
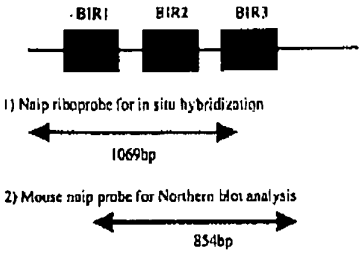
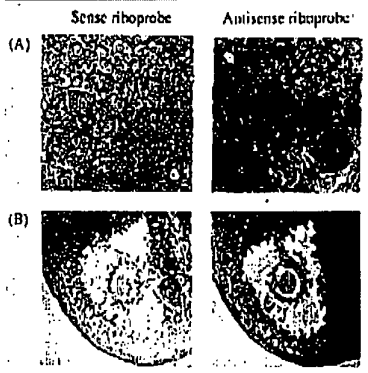


Fig. 1. Folliculor development in the mouse ovary

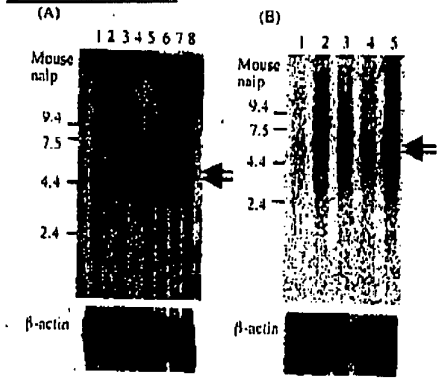
[Drawing 2]



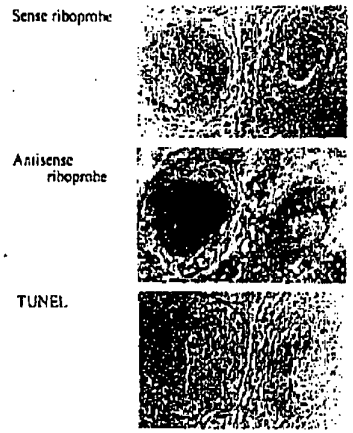
[Drawing 3]



[Drawing 4]



[Drawing 5]



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15/09	ＺＮＡ	15/00	ＺＮＡＡ

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(54)【発明の名称】　超過排卵動物と超過排卵方法

(57)【要約】

【課題】　卵胞の退行・閉鎖を抑制するＮＡＩＰ遺伝子の発現を人為的に制御可能な動物個体と、ＮＡＩＰ遺伝子の発現を制御する方法を提供する。

【解決手段】　プロモーター配列と配列番号１または２のＤＮＡ配列とを含むＤＮＡ断片を導入した分化全能性細胞を個体へと発生させたトランスジェニック非ヒト動物およびその子孫動物であって、体細胞染色体中に上記ＤＮＡ断片を保有することを特徴とする超過排卵動物と、この動物の導入遺伝子を発現させて配列番号３または４のアミノ酸配列を有するタンパク質を産生させることを特徴とする超過排卵方法。

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【特許請求の範囲】

【請求項１】　プロモーター配列と配列番号１または２のＤＮＡ配列とを含むＤＮＡ断片を導入した分化全能性細胞を個体へと発生させたトランスジェニック非ヒト動物およびその子孫動物であって、体細胞染色体中に上記ＤＮＡ断片を保有することを特徴とする超過排卵動物。

【請求項２】　プロモーター配列が、性腺刺激ホルモンのレセプター遺伝子のプロモーター配列である請求項１の超過排卵動物。

【請求項３】　請求項１記載の超過排卵動物の排卵を人為的に促進させる方法であって、プロモーター配列の転写制御因子を動物に投与し、配列番号３または４のアミノ酸配列を有するタンパク質を発現させることを特徴とする超過排卵方法。

【請求項４】　請求項２記載の超過排卵動物の排卵を人為的に促進させる方法であって、性腺刺激ホルモンを動物に投与し、配列番号３または４のアミノ酸配列を有するタンパク質を発現させることを特徴とする超過排卵方法。

【請求項５】　プロモーター配列と配列番号１または２のＤＮＡ配列とを含むＤＮＡ断片を含有する組換えベクターにより形質転換した卵母細胞を卵巣中に導入された超過排卵動物。

【請求項６】　プロモーター配列が、性腺刺激ホルモンのレセプター遺伝子のプロモーター配列である請求項５の超過排卵動物。

【請求項７】　請求項５記載の超過排卵動物の排卵を人為的に促進させる方法であって、プロモーター配列の転写制御因子を動物に投与し、配列番号３または４のアミノ酸配列を有するタンパク質を発現させることを特徴とする超過排卵方法。

【請求項８】　請求項６記載の超過排卵動物の排卵を人為的に促進させる方法であって、性腺刺激ホルモンを動物に投与し、配列番号３または４のアミノ酸配列を有するタンパク質を発現させることを特徴とする超過排卵方法。

【請求項９】　ナイーブ動物個体の排卵を人為的に促進させる方法であって、タンパク質リン酸化酵素抑制因子またはスタウロスポリンもしくはそれらの誘導体を動物個体に投与することによって、配列番号３または４のアミノ酸配列を有するタンパク質を発現させることを特徴とする超過排卵方法。

【請求項１０】　プロモーター配列と配列番号１のＤＮＡ配列とを含むＤＮＡ断片を含有した組換えベクター。

【請求項１１】　性腺刺激ホルモンのレセプター遺伝子のプロモーター配列と配列番号１のＤＮＡ配列とを含むＤＮＡ断片を含有した組換えベクター。

【請求項１２】　プロモーター配列と配列番号２のＤＮＡ配列とを含むＤＮＡ断片を含有した組換えベクター。

【請求項１３】　性腺刺激ホルモンのレセプター遺伝子のプロモーター配列と配列番号２のＤＮＡ配列とを含むＤＮＡ断片を含有した組換えベクター。

2

【請求項１４】　請求項１または２記載の超過排卵動物より単離された細胞。

【請求項１５】　細胞が、生殖細胞である請求項１４の細胞。

【請求項１６】　プロモーター配列と配列番号１または２のＤＮＡ配列とを含むＤＮＡ断片を含有する組換えベクターにより形質転換した卵母細胞。

【請求項１７】　プロモーター配列が、性腺刺激ホルモンのレセプター遺伝子のプロモーター配列である請求項１６の卵母細胞。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】この発明は、成熟卵子の排卵数を増加させることのできる遺伝子導入超過排卵動物と、この超過排卵動物またはナイーブ動物の排卵数を人為的に制御する方法に関するものである。

【０００２】

【従来の技術】哺乳動物の雌個体の卵巣では、胎生期または生後に多くの原始卵胞(primodialfollicles)が形成される。この原始卵胞は、将来卵子となる生殖細胞である卵母細胞とそれを取り巻く顆粒膜細胞で構成されるもので、これらの原始卵胞は個体の生涯を通じて性周期ごとに決まった数が一次卵胞(primary follicles)、二次卵胞(secondary follicles)、胞状卵胞(antral follicles あるいはvesicularfollicles)、そしてグラーフ卵胞(graafian follicles)へと発育していき、最後には卵母細胞が成熟して排卵(ovulation)に至る過程を踏む。しかし、出生時の原始卵胞の数は動物種によって限られており、図１に示したように、極わずかの原始卵胞が排卵過程に達するのみであり、一部は休止状態で終わるものの、99.9% の原始卵胞は、発育途上で「卵胞閉鎖」(atresia)と言われる過程を介して退行していく。

【０００３】卵胞が退行する要因としては、年齢、繁殖周期、妊娠、泌乳、卵巣内外のホルモンバランス、栄養、局所貧血等が挙げられているものの、その詳細な分子メカニズムは長い間不明であった。近年、卵胞閉鎖の際に観られる形態学的、生化学的、組織学的変化の観察から、閉鎖卵胞の顆粒膜細胞に核クロマチンの濃縮や核の断片化が認められ、卵胞の閉鎖とアポトーシスの関係が示唆されている。細菌の研究から、性腺刺激ホルモンがラットの閉鎖卵胞におけるアポトーシスを抑制すること(Tilly et al.,「文献名、雑誌名、vol. page」 1992; Chun et al.,Endocrinology 135: 1845-1853, 1994; Tilly et al., Endocrinology 136: 1394-14023, 1995; Tilly and Tilly, Endocrinology 136: 242-252, 1995)、またその抑制作用の一部は卵胞内の成長ホルモンを介していること(Tilly et al.,Mol. Endocrinol. 6: 1942-1950, 1992; Chun et al., Endocrinology 135: 1

845-1853, 1994; Tilly et al., Endocrinology 136: 1394-14023, 1995)が明らかになっている。さらに、アポトーシスが卵胞の選択機構の要となっている可能性の裏付けとして、性腺刺激ホルモンに依存した顆粒膜細胞内の活性酵素(Tilly and Tilly, Endocrinology 136: 242-252, 1995)、ｐ５３等の癌抑制遺伝子(Tilly et al., Endocrinology 136: 1394-14023)、ced-3/インターロイキン-1転換酵素(ICE: interleukin-1 β converting enzyme) 関連遺伝子(Flaws et al.,Endocrinology 136: 5042-5053) の変化が報告されている。

【０００４】しかし、これらの要因が生殖細胞である卵母細胞を含む卵巣内卵胞の生存・選択を制御する機構においていかなる機能を果たしているか、さらにアポトーシスを抑制する因子がそれらのどのように関係しているかについては、詳細な解明はなされていなかった。ところが、ごく最近、アポトーシスを抑制し、細胞の延命機能を有するBcl-２関連遺伝子産物がラット卵巣内卵胞で発現していることが報告されたが (Tilly et al., Endocrinology 136:232-241, 1995)、遺伝子ターゲッティングによってBcl-２遺伝子を欠損させたマウスの観察から、原始卵胞の生存性にのみＢcl- ２が関与していることが示唆された (Ratts et al., Endocrinology 136:3665-3668,1995)。このことから、原始卵胞から発育した一次卵胞、二次卵胞、そして胞状卵胞における卵巣内卵胞の退行・閉鎖の過程では、Bcl-２関連遺伝子産物とは別のアポトーシス抑制因子が関与している可能性が想定される。

【０００５】一方、近年の分子生物学的手法の発達に伴い、その手法の一つであるポジショナルクローニングによって、家族性の遺伝病である脊椎性筋萎縮症候群(spinalmuscular atropy : ＳＭＡ) の原因遺伝子として、神経細胞アポトーシス抑制タンパク質(nural apoptotic inhibitory protein:ＮＡＩＰ) が単離された (Royet al., Cell 80:167-178, 1995)。さらに、このＮＡＩＰ遺伝子を種々の培養細胞に導入し、アポトーシスを誘起させる刺激を細胞に与えたところ、その細胞死が抑制されることが明らかになった(Liston et al., Nature 379:349-353, 1996) 。これらの結果から、ＮＡＩＰはBcl-２関連遺伝子産物の関与だけでは説明できないアポトーシスの制御機構において細胞延命的な機能を持つ因子である可能性が示唆された。

【０００６】生体内において、アポトーシスといわれる生理的細胞死は、無数の細胞から構成される生体の恒常性を保つ上で、不要な細胞を排除する機構として必須な現象である。動物個体におけるＮＡＩＰの発現は、神経細胞のアポトーシスを抑制するだけではなく、卵胞の退行・閉鎖を制御し、その動物種にプログラムされた排卵数を一定に保つようにも機能していると考えられる。従って、このＮＡＩＰ遺伝子の発現を人為的にコントロールすることが可能になれば、ヒトにおける不妊療法をはじめとして、排卵誘発剤処理にもかかわらず排卵数が少数である有用動物（牛、馬等の家畜動物など）を効率よく生産することが可能となる。

【０００７】この発明は、以上のとおりの事情に鑑みてなされたものであり、外来性ＮＡＩＰ遺伝子の全ｃＤＮＡ配列を保有する超過排卵動物を提供することを目的としている。またこの発明は、上記動物の排卵数を人為的に促進させる方法を提供することを目的としている。

【０００８】さらにこの発明は、ヒトを含めたナイーブ動物個体（外来性遺伝子が導入されていない動物個体）の排卵数を人為的に増加させる方法を提供することを目的としてもいる。

【０００９】

【課題を解決するための手段】この発明は、上記の課題を解決するものとして、、プロモーター配列と配列番号１または２のＤＮＡ配列とを含むＤＮＡ断片を導入した分化全能性細胞を個体へと発生させたトランスジェニック非ヒト動物およびその子孫動物であって、体細胞染色体中に上記ＤＮＡ断片を保有することを特徴とする超過排卵動物（請求項１）を提供する。

【００１０】またこの発明は、プロモーター配列と配列番号１または２のＤＮＡ配列とを含むＤＮＡ断片を含有する組換えベクターにより形質転換した卵母細胞を卵巣中に導入された超過排卵動物（請求項５）を提供する。なおこれらの超過排卵動物においては、上記プロモーター配列が、性腺刺激ホルモンのレセプター遺伝子のプロモーター配列であること（請求項２および６）を好ましい態様としている。

【００１１】さらにこの発明は、上記の超過排卵動物（請求項１および５）の排卵を人為的に促進させる方法であって、プロモーター配列の転写制御因子を動物に投与し、配列番号３または４のアミノ酸配列を有するタンパク質を発現させることを特徴とする超過排卵方法（請求項３および７）を提供する。さらにまたこの発明は、プロモーター配列が性腺刺激ホルモンのレセプター遺伝子のプロモーター配列である超過排卵動物（請求項２および６）の排卵を人為的に促進させる方法であって、性腺刺激ホルモンを動物に投与し、配列番号３または４のアミノ酸配列を有するタンパク質を発現させることを特徴とする超過排卵方法（請求項４および８）を提供する。

【００１２】また、さらにこの発明は、ナイーブ動物個体の排卵を人為的に促進させる方法であって、タンパク質リン酸化酵素抑制因子またはスタウロスポリンもしくはそれらの誘導体を動物個体に投与することによって、配列番号３または４のアミノ酸配列を有するタンパク質を発現させることを特徴とする超過排卵方法（請求項９）を提供する。

【００１３】以上の発明の他、この発明は、プロモーター配列と配列番号１または２のＤＮＡ配列とを含むＤＮ

Ａ断片を含有した組換えベクター（請求項１０および１２）、性腺刺激ホルモンのレセプター遺伝子のプロモーター配列と配列番号１または２のＤＮＡ配列とを含むＤＮＡ断片を含有した組換えベクター（請求項１１および１３）、上記のトランスジェニック非ヒト動物より単離された細胞（請求項１４）、プロモーター配列と配列番号１または２のＤＮＡ配列とを含むＤＮＡ断片を含有する組換えベクターにより形質転換した卵母細胞（請求項１６）をそれぞれ提供する。

【００１４】以下、この発明の実施の形態について詳しく説明する。

【００１５】

【発明の実施の形態】この発明において、卵胞の退行・閉鎖抑制の手段として使用する遺伝子は、ＳＭＡの原因遺伝子としてヒト第５染色体長腕１３．１領域（５ｑ１３．１）より単離されたＮＡＩＰ遺伝子であり、その全長ｃＤＮＡは、配列番号１または配列番号２の塩基配列を有している。このようなｃＤＮＡは、例えば配列番号１または２の１部配列をプローブとして、既存の各種動物由来のｃＤＮＡライブラリから単離することができ、あるいは配列番号１または２の一部配列をプライマーとしてＰＣＲ増幅して得ることができる。また、動物個体に導入するｃＤＮＡは、配列番号１または２のＤＮＡ配列の全長でもよく、あるいは、そのコード領域部分でもよい。そして、この発明の超過排卵動物の作成においては、このｃＤＮＡ配列と、その上流にプロモーター配列を連結したＤＮＡ断片を調製し、このＤＮＡ断片を導入した分化全能性細胞を個体へと発生させたトランスジェニック動物を作成する。

【００１６】プロモーター配列は、このｃＤＮＡ配列を卵巣内で発現させるために、卵巣特異的に存在する物質を転写制御因子とする遺伝子のプロモーター配列を用いるのが好ましく、特に、卵胞刺激ホルモンや黄体ホルモン等の性腺刺激ホルモンのレセプター遺伝子のプロモーター配列が好ましい。あるいは、使用する動物種に内在しない物質を転写制御因子とする外来遺伝子のプロモーター配列を使用することもできる。この場合は、導入したｃＤＮＡが自発的には発現せず、外部からその転写制御因子を投与した場合にのみ発現させることができる。

【００１７】例えば、この発明のトランスジェニック動物は以下の方法で作成することができる。プロモーター配列と配列番号１または２のＤＮＡ配列（またはその一部配列）とを、各々の解読枠を一致させて転結してＤＮＡ断片を調製し、このＤＮＡ断片を非ヒト哺乳動物の分化全能性細胞に導入する。対象となる動物は、例えばウシ、ブタ、ウマ、ヒツジ等の有用家畜であり、あるいはイヌやネコ、霊長類等の愛玩動物、実験用動物などである。また、分化全能性細胞としては、受精卵や初期胚のほか、ＥＳ細胞のような培養細胞を使用することができる。これらの分化全能性細胞へのＤＮＡ断片の注入は、

公知の方法、すなわち静電パルス法、リポソーム法、リン酸カルシウム法、マイクロインジェクション法等を用いることができる。次に、ＤＮＡ断片を注入した細胞を仮親の卵管に移植し、個体まで発生した動物を出生させて飼育する。そして、体細胞を取り出し、この細胞中のＤＮＡをサザンブロット分析して導入したＤＮＡ断片の存在を確認する。ＤＮＡ断片の染色体への組み込みが確認された個体を初代（Founder）とすれば、この導入ＤＮＡ断片はその子孫の５０％に伝達され、この発明の超過排卵動物を継続的に生産することができる。

【００１８】そして、このようにして作成した超過排卵動物は、その全細胞の染色体に導入ＤＮＡ断片を有しており、プロモーター配列に特異的な転写制御因子の存在によってＮＡＩＰ遺伝子のｃＤＮＡを発現させる。プロモーター配列が、卵胞刺激ホルモンのレセプター遺伝子のプロモーターである場合には、ｃＤＮＡは、そのホルモンが存在する卵巣の顆粒膜細胞でのみ特異的に発現し、卵胞の退行・閉鎖を抑制する。もちろん、動物個体には内在性のＮＡＩＰ遺伝子が存在して排卵数のコントロールを行っているが、この発明の超過排卵動物は、導入した外来性のＮＡＩＰ遺伝子ｃＤＮＡによって卵胞の退行・閉鎖抑制因子を多量に発現するため、ナイーブな動物に比べて多量の排卵が可能である。また、プロモーターに特異的な転写制御因子を投与することによって、超過排卵を人為的に制御することもできる。

【００１９】一方、この発明の超過排卵動物は、動物個体の卵巣内に上記ＤＮＡ断片を直接的に導入することによっても作成することができる。すなわち、ＤＮＡ断片を組み込んだ組換えベクター（アデノウイルスベクター、レトロウイルスベクター等）を物理的な方法によって動物個体の卵巣に移植する。あるいは、上記組換えベクターによって形質転換した卵母細胞を卵巣に移植する。このような動物も、卵巣内においてＮＡＩＰ遺伝子がナイーブ動物よりも多量発現し、超過排卵となる。また、ＮＡＩＰ遺伝子ｃＤＮＡに連結したプロモーター配列の転写制御因子を投与することによって、その超過排卵をコントロールすることが可能である。

【００２０】さらにまた、この発明は、ナイーブ動物個体の排卵を人為的に促進させる超過排卵方法も提供する。すなわち、全ての動物個体は、そのゲノム中にＮＡＩＰ遺伝子を備えており、この内在性の遺伝子は、タンパク質リン酸化酵素抑制因子（例えば、免疫抑制剤ＦＫ５０６等）やスタウロスポリン（Ｋ２５２Ａ等）、もしくはこれらの誘導体を全身投与することによって一過性に発現させることができる。これによって、ヒトを含めた動物個体において、排卵誘発剤等を用いることなく大量の排卵が可能となり、安全かつ確実な不妊療法が提供される。

【００２１】次に、ＮＡＩＰ遺伝子が確かに卵胞の退行・閉鎖抑制に関与する遺伝子であることを確認した実験

結果を示し、この発明の有効性を説明する。

（１）材料および方法

動物および性腺刺激ホルモン処理

ＩＣＲ系雌マウス（日本クレア（株）より購入）を、明期１２時間(5:00 ～17:00)、湿度ならびに温度を一定に調節した環境下で飼育し、３週令の時点で過排卵処理した。すなわち、５ＩＵの妊馬血清性腺刺激ホルモン（pregnant mare serum gonadotropin, ＰＭＳＧ）を腹腔内投与し、４８時間後に５ＩＵのヒト絨毛性性腺刺激ホルモン(human chorionic gonadotropin, ｈＣＧ）を同様に腹腔内投与した。

in situ hybridization

ブアン固定したマウス卵巣を脱水処理したのち、パラフィン包埋し、厚さ５～６μｍの切片に薄切後、シランコート処理したスライドグラスに拾つた。乾燥させたのち、常法に従って脱パラフィンならび親水操作を行った組織切片を、0.3%Triton-X（2 分間）、0.2 %HCl （20分間）、20μg/mlのプロテアーゼＫ（２０分間）で処理した。さらに、4 %の新鮮なパラフォルムアルデヒドに５分間浸漬して再固定させた後、0.2 %グリシン中で１時間処理して残存アルデヒトを中和し、プレ・ハイブリダイゼーションとして50%フォルムアミドで２時間処理した。

【００２２】ハイブリダイゼージョンに用いるＮＡＩＰのアンチセンスおよびセンスＲＮＡプローブには、ヒトnaip遺伝子 (Roy et al., Cell 80:167-178, 1995)の1955 塩基から1263塩基までのＢＩＲ(baculoviral inhibition of apoptosis protein repeat)領域のＢＩＲ１、ＢＩＲ２およびＢＩＲ３の一部を含む遺伝子断片（図２）をpBluscriptにサブクローニングしたベクターを用いて、ベーリンガーマンハイム社のジゴキシゲニン（Digoxigenin ：ＤＩＧ）- ＲＮＡ labeling Kit を利用してin vitro転写させることによって生成したＤＩＧ標識ＲＮＡプローブを使用した。なお、このプローブについて、ＩＡＰ(inhibitor of apoptosis protein)関連遺伝子であるマウスc-ＩＡＰ１とのホモロジーは30～40%であり、交差性が低いことが判っている。ハイブリダイゼーションは、50%フォルムアミド、10%硫酸デキストラン、１Ｘデンハルト溶液、100 μg/ml一本鎖サケ精子ＤＮＡ、100μg/ml大腸菌ｔＲＮＡ、10 mMディチオステオールの混合液に、予め65℃、５分間の処理で変性させたＲＮＡプローブを加えた溶液を用いて、モイスチャーチャンバー中で50℃の条件下16～20時間行った。次に、未反応のＲＮＡプローブを組織標本から除くために以下の洗浄操作を行った。まず、４ＸＳＳＣ中で20分間洗浄し、20μg/mlのＲＮａｓｅ中で37℃、30分間処理して洗浄した後、さらに68℃の条件下２ＸＳＳＣおよび 0.2ＸＳＳＣ中で各１時間洗浄した。

【００２３】免疫組織化学反応による検出では、ベーリンガーマンハイム社のアルカリフォスファターゼ標識ＤＩＧ抗体を反応させた後、5-bromo-4-chloro-3-indoyl-phosphate-nitroblue tetrazolium （ＢＣＩＰ／ＮＢＴ）を基質として４℃の条件下で発色させることでＮＡＩＰ遺伝子のＲＮＡの存在を示すシグナルを検出した。

アポトーシスの組織化学的検出

マウス卵巣のブアン固定、パラフィン包埋、ならびに組織切片の作成については、前述の方法に準じた。形態学的なアポトーシスの検出は、染色体ＤＮＡのヌクレソーム単位での断片化を組織化学的に検出する方法であるTerminal Deoxynucleotidly Transferase (TdT)-mediated dUTP-biotin nick end labeling（ＴＵＮＥＬ）法を利用した。ＴＵＮＥＬアッセイには、ＭＥＢＳＴＡＩＮ Kit（医学生物学研究所）を基本的に使用し、まず組織切片をプロテアーゼ処理した後、核における断片化ＤＮＡの3'OH末端部分にビオチン化dUTPを取り込ませた後、ストレプトアビジンーＨＲＰ(horseradish peroxidase)を加えて結合させ、基質としてＤＡＢ(diaminobenzidine)を使って発色させて断片化ＤＮＡを検出した。

顆粒膜細胞の単離および培養

３週令のＩＣＲ幼若雌マウスより卵巣を摘出し、濾紙上で脂肪組織や血液等を取り除き、イーグル最小必須培地(minimum essential medium:ＭＥＭ) で洗浄後に実験に供試した。卵母細胞を含む顆粒膜細胞の細胞塊の単離および培養は、Eppig ら (Biol. Reprod., 41:268-276, 1989)とHiranoら(J. Exp.Zool., 267:543-547, 1993) が報告した方法にほぼ準じて行った。先ず、2mg/mlのコラゲナーゼ（細胞分散用、和光純薬）を添加したイーグルＭＥＭに摘出した卵巣を浸漬し30分間処理した。洗浄後、パスツールピペットでピペッティングを繰り返して、卵母細胞を含む顆粒細胞の細胞塊を単離した。単離した細胞塊は、寒天でコートした培養皿中の4mg/mlウシ血清アルブミンおよび 2mMヒポキサンチンで培養した。培養開始10日後に、これら卵母細胞を含む顆粒膜細胞の細胞塊を100ng/mlの卵胞刺激ホルモン（fillicle-stimulating hormone: ＦＳＨ、シグマ)を含むイーグルＭＥＭに移し、18時間後に卵母細胞を含む顆粒膜細胞の細胞塊を採取し、ＮＡＩＰ遺伝子発現の解析に供した。

ＲＮＡ抽出と逆転写酵素ＰＣＲ（ＲＴ－ＰＣＲ）およびノーザンブロット解析

卵巣からの全ＲＮＡの抽出は、ＡＧＰＣ(acid guanidium thiocyanate-phenol-chloroform)法(Anal.Biochem. 162:156-159, 1987) によって行った。抽出した全ＲＮＡは、ＤＮaseI処理することで混入したゲノムＤＮＡを消化したのち、定量して実験に供した。

【００２４】ＲＴ－ＰＣＲ解析では、１μｇ の全ＲＮＡからＡＭＶＸＬ ＲＴase （宝酒造）によつてｃＤＮＡを合成し、その一部を使ってマウスnaipのｃＤＮＡを増幅した。増幅に使用したプライマーは、5'-CACAGGGGTGAAACTTGGGGTTCAG-3' および5'-CACCTGTGGTTTCCATGGCTTCTGG-3' であり、反応条件は、熱変性94℃を５分間した

後、熱変性94℃を１分間、アニーリング60℃を１分間、ＤＮＡ合成72℃を２分間のサイクルを40回繰り退した。増幅したＲＴ－ＰＣＲ産物は、２％アガロースゲルで電気泳動して分離、検出した。

【００２５】ノーザンブロット解析では、全ＲＮＡよりOligotex-dT<Super>（宝酒造）を使って精製した６～８μｇのポリ（Ａ）$^+$ ＲＮＡを使用した。ホルムアミドの存在下でアガロース電気泳動を分離した後、ナイロンフィルターであるybondN$^+$（アマシャム）にトランスファーした。翌日、乾燥させたナイロンフィルターに紫外線照射して、フィルター上のＲＮＡを固定化した。プレハイブリダイゼーションおよびハイブリダイゼーションでは、ExpressHyb hybridization solution（クローンテック）を使用した。先ず、２～３時間のプレハイブリダイゼーションの後、^{32}Ｐ-ｄＣＴＰ標識したマウスｎnaipｃＤＮＡプローブを加えて一晩ハイブリダイゼーションを行った。なお、プローブに用いた遺伝子断片は、マウスnaip遺伝子（Robertson ら、未発表データ）の473 塩基から1326塩基までのＢＩＲ１の一部とＢＩＲ２およびＢＩＲ３を含む854 塩基の長さの遺伝子断片である（図２）。やはり、ＩＡＰ関連遺伝子であるマウスc-ＩＡＰ１とのホモロジーは30～40％であり、交差性が低いことが判っている。ハイブリダイゼーションの洗浄は、２ＸＳＳＣと0.05％ＳＤＳの水溶液中で室温、20分間を２回、次に0.１ＸＳＳＣと0.1 ％ＤＳＤの水溶液中で68℃、20 分間を２回することで行った。洗浄したフィルターは、イメージングプレート（フジ）でオートラジオグラフィーを行い、ＢＡＳ－2000でシグナルを検出し、定量解析を行った。

（２）結果

卵巣内卵胞の発育に伴うＮＡＩＰ遺伝子の発現

種々の週令の雌マウスより採取した卵巣におけるＮＡＩＰ遺伝子の発現を、そのｃＤＮＡに対する特異的リボプローブを用いたin situ hybridization によって調べることで、卵巣内卵胞の発育に伴うＮＡＩＰ遺伝子の発現を検討した。

【００２６】出生直後の２日令マウス卵巣で観察される卵巣内卵胞像は、一層の扁平な顆粒膜細胞で卵母細胞が囲まれた原始卵胞、あるいは立方状になった顆粒膜細胞と卵母細胞、そして外周の基底膜で構成される一次卵胞の２つの卵胞像であるが、原始卵胞ではセンスおよびアンチセンスのリボプローブをハイブリダイズさせた場合とも差が見られなかった。一方、より発育した卵母細胞を持つ一次卵胞の顆粒膜細胞においてはアンチセンスリボプローブを使った際により強いシグナルが観察された（図３（A））。次に、性周期を規則的に繰り返す12週令の成熟したマウス卵巣においてＮＡＩＰ遺伝子の発現を調べたところ、排卵直前の胞状卵胞であるグラーフ卵胞の顆粒膜細胞および卵母細胞を取り巻く卵丘細胞においてもＮＡＩＰ遺伝子の発現が認められた（図３（B））。

【００２７】以上の結果から、ＮＡＩＰ遺伝子は、一次卵胞から排卵直前のグラーフ卵胞までの顆粒膜細胞で発現していることが確認された。

ＮＡＩＰ遺伝子の組織特異的発現

卵巣を含むマウスの各組織におけるＮＡＩＰ遺伝子の発現をマウスnaipｃＤＮＡをプローブとしてノーザンブロット解析によって調べた。

【００２８】ＮＡＩＰ遺伝子は、卵巣を含めマウス各組織では２つの長さをもつ転写産物として発現している。先ず、２μｇのポリ（A)$^+$ ＲＮＡを結合させたマウスmultiple-tissue northernblot（ＭＴＮ blot 、クローンテック）を使って、マウスの主な組織におけるＮＡＩＰ遺伝子の発現を検討したところ、脾臓、肺、肝臓、心臓で強いシグナルが観られるものの、組織特異性はなく、ほぼ全組織で発現が認められた（図４（A））。一方、卵巣での発現については、２日令、３週令、12週令、分娩３日目、および18週令の種々の雌マウスより採取した卵巣由来の８μｇのポリ（A)$^+$ ＲＮＡをブロットさせたフィルターを使ったところ、一次、二次卵胞や胞状卵胞の割合が多い性周期を繰り返す成熟した12週令および18週令の雌マウスで強いＮＡＩＰ遺伝子の発現が観察された。ただし、それぞれ原始卵胞や黄体が多くを占める２日令や分娩３日めの雌マウスの卵巣においてはＮＡＩＰ遺伝子の発現は弱かった。

【００２９】以上のことから、卵巣内におけるＮＡＩＰ遺伝子の発現は、卵胞の発達過程と相関関係にあることが確認された。

卵胞内におけるＮＡＩＰ遺伝子発現の局在性

性腺刺激ホルモンで過排卵処理を施した３週令の雌マウスの卵巣において、ＮＡＩＰ遺伝子の経時的な発現を調べた。卵胞刺激ホルモン（ＦＳＨ）としての作用をもつＰＭＳＧ投与48時間後の卵巣でのＮＡＩＰ遺伝子の発現量は、投与前に比較して約1.6 倍であり、さらに黄体ホルモン（Lutinating hormone, ＬＨ）作用を有するｈＣＧの投与７時間後には、投与前と比較して約2.4 倍強い発現が観察された。つぎに、卵巣より単離した卵母細胞を含む顆粒膜細胞の細胞塊におけるＮＡＩＰ遺伝子の発現をＲＴ－ＰＣＲによって検出したところ、卵母細胞では発現が認めらず、顆粒膜細胞でのみ発現していることが示された。さらに、ＰＣＲによる増幅のため絶対的な定量性はないが、性腺刺激ホルモンによってＮＡＩＰ遺伝子の発現シグナルが強くなる傾向が観察された。

【００３０】以上の結果から、ＮＡＩＰ遺伝子の発現は卵胞の顆粒膜細胞に局在し、ＦＳＨなどの性腺刺激ホルモンによってその発現が増強されることが確認された。

卵胞閉鎖とＮＡＩＰ遺伝子発現との関係

卵巣の連続組織切片上において、in situ hybridization によって検出されるＮＡＩＰ遺伝子の発現とＴＵＮＥＬ法によって認められるアポトーシスを比較検討した。その結果、図５に示したように、ＮＡＩＰ遺伝子が強く

発現している卵胞の顆粒膜細胞ではアポトーシスが観察されなかった。一方、卵母細胞の変形を特徴とする閉鎖卵胞では、細胞死した顆粒膜細胞が観察され、このような卵胞ではＮＡＩＰ遺伝子の発現は、微弱であるか、またはほとんど観察されなかった。

【００３１】以上のとおり、閉鎖した卵胞ではＮＡＩＰ遺伝子が発現していないことから、ＮＡＩＰ遺伝子は卵巣内卵胞においてアポトーシス抑制因子として機能していることが確認された。

【００３２】

【発明の効果】以上詳しく説明したとおり、この発明によって、卵胞の退行・閉鎖を抑制するＮＡＩＰ遺伝子を導入した超過排卵動物と、ＮＡＩＰ遺伝子の発現を制御することによって排卵を人為的に促進する方法が提供される。これによって、新たな不妊療法の開発が可能となり、有用動物の生産性も向上させることができる。

【００３３】

【配列表】

配列番号：１
配列の長さ：5984
配列の型：核酸
鎖の数：二本鎖
トポロジー：直鎖状
配列の種類：cDNA to mRNA
起源
生物名：ヒト

配列

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ACAAAAGGTC CTGTGCTCAC CTGGGACCCT TCTGGACGTT GCCCTGTGTT CCTCTTCGCC   60
TGCCTGTTCA TCTACGACGA ACCCCGGGTA TTGACCCCAG ACAACAATGC CACTTCATAT  120
TGGGGACTTC GTCTGGGATT CCAAGGTGCA TTCATTGCAA AGTTCCTTAA ATATTTTCTC  180
ACTGCTTCCT ACTAAAGGAC GGACAGAGCA TTTGTTCTTC AGCCACATAC TTTCCTTCCA  240
CTGGCCAGCA TTCTCCTCTA TTAGACTAGA ACTGTGGATA AACCTCAGAA AATGGCCACC  300
CAGCAGAAAG CCTCTGACGA GAGGATCTCC CAGTTTGATC ACAATTTGCT GCCAGAGCTG  360
TCTGCTCTTC TGGGCCTAGA TGCAGTTCAG TTGGCAAAGG AACTAGAAGA AGAGGAGCAG  420
AAGGAGCGAG CAAAAATGCA GAAAGGCTAC AACTCTCAAA TGCGCAGTGA AGCAAAAAGG  480
TTAAAGACTT TTGTGACTTA TGAGCCGTAC AGCTCATGGA TACCACAGGA GATGGCGGCC  540
GCTGGGTTTT ACTTCACTGG GGTAAAATCT GGGATTCAGT GCTTCTGCTG TAGCCTAATC  600
CTCTTTGGTG CCGGCCTCAC GAGACTCCCC ATAGAAGACC ACAAGAGGTT TCATCCAGAT  660
TGTGGGTTCC TTTTGAACAA GGATGTTGGT AACATTGCCA AGTACGACAT AAGGGTGAAG  720
AATCTGAAGA GCAGGCTGAG AGGAGGTAAA ATGAGGTACC AAGAAGAGGA GGCTAGACTT  780
GCATCCTTCA GGAACTGGCC ATTTTATGTC CAAGGGATAT CCCCTTGTGT GCTCTCAGAG  840
GCTGGCTTTG TCTTTACAGG TAAACAGGAC ACGGTACAGT GTTTTTCCTG TGGTGGATGT  900
TTAGGAAATT GGGAAGAAGG AGATGATCCT TGGAAGGAAC ATGCCAAATG GTTCCCCAAA  960
TGTGAATTTC TTCGGAGTAA GAAATCCTCA GAGGAAATTA CCCAGTATAT TCAAAGCTAC 1020
AAGGGATTTG TTGACATAAC GGGAGAACAT TTTGTGAATT CCTGGGTCCA GAGAGAATTA 1080
CCTATGGCAT CAGCTTATTG CAATGACAGC ATCTTTGCTT ACGAAGAACT ACGGCTGGAC 1140
TCTTTTAAGG ACTGGCCCCG GGAATCAGCT GTGGGAGTTG CAGCACTGGC CAAAGCAGGT 1200
CTTTTCTACA CAGGTATAAA GGACATCGTC CAGTGCTTTT CCTGTGGAGG GTGTTTAGAG 1260
AAATGGCAGG AAGGTGATGA CCCATTAGAC GATCACACCA GATGTTTTCC CAATTGTCCA 1320
TTTCTCCAAA ATATGAAGTC CTCTGCGGAA GTGACTCCAG ACCTTCAGAG CCGTGGTGAA 1380
CTTTGTGAAT TACTGGAAAC CACAAGTGAA AGCAATCTTG AAGATTCAAT AGCAGTTGGT 1440
CCTATAGTGC CAGAAATGGC ACAGGGTGAA GCCCAGTGGT TTCAAGAGGC AAAGAATCTG 1500
AATGAGCAGC TGAGAGCAGC TTATACCAGC GCCAGTTTCC GCCACATGTC TTTGCTTGAT 1560
ATCTCTTCCG ATCTGGCCAC GGACCACTTG CTGGGCTGTG ATCTGTCTAT TGCTTCAAAA 1620
CACATCAGCA AACCTGTGCA AGAACCTCTG GTGCTGCCTG AGGTCTTTGG CAACTTGAAC 1680
TCTGTCATGT GTGTGGAGGG TGAAGCTGGA AGTGGAAAGA CGGTCCTCCT GAAGAAAATA 1740
GCTTTTCTGT GGGCATCTGG ATGCTGTCCC CTGTTAAACA GGTTCCAGCT GGTTTTCTAC 1800
CTCTCCCTTA GTTCCACCAG ACCAGACGAG GGGCTGGCCA GTATCATCTG TGACCAGCTC 1860
CTAGAGAAAG AAGGATCTGT TACTGAAATG TGCATGAGGA ACATTATCCA GCAGTTAAAG 1920
AATCAGGTCT TATTCCTTTT AGATGACTAC AAAGAAATAT GTTCAATCCC TCAAGTCATA 1980
GGAAAACTGA TTCAAAAAAA CCACTTATCC CGGACCTGCC TATTGATTGC TGTCCGTACA 2040
AACAGGGCCA GGGACATCCG CCGATACCTA GAGACCATTC TAGAGATCAA AGCATTTCCC 2100
TTTTATAATA CTGTCTGTAT ATTACGGAAG CTCTTTTCAC ATAATATGAC TCGTCTGCGA 2160
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AAGTTTATGG TTTACTTTGG AAAGAACCAA AGTTTGCAGA AGATACAGAA AACTCCTCTC 2220
TTTGTGGCGG CGATCTGTGC TCATTGGTTT CAGTATCCTT TTGACCCATC CTTTGATGAT 2280
GTGGCTGTTT TCAAGTCCTA TATGGAACGC CTTTCCTTAA GGAACAAAGC GACAGCTGAA 2340
ATTCTCAAAG CAACTGTGTC CTCCTGTGGT GAGCTGGCCT TGAAAGGGTT TTTTTCATGT 2400
TGCTTTGAGT TTAATGATGA TGATCTCGCA GAAGCAGGGG TTGATGAAGA TGAAGATCTA 2460
ACCATGTGCT TGATGAGCAA ATTTACAGCC CAGAGACTAA GACCATTCTA CCGGTTTTTA 2520
AGTCCTGCCT TCCAAGAATT TCTTGCGGGG ATGAGGCTGA TTGAACTCCT GGATTCAGAT 2580
AGGCAGGAAC ATCAAGATTT GGGACTGTAT CATTTGAAAC AAATCAACTC ACCCATGATG 2640
ACTGTAAGCG CCTACAACAA TTTTTTGAAC TATGTCTCCA GCCTCCCTTC AACAAAAGCA 2700
GGGCCCAAAA TTGTGTCTCA TTTGCTCCAT TTAGTGGATA ACAAAGAGTC ATTGGAGAAT 2760
ATATCTGAAA ATGATGACTA CTTAAAGCAC CAGCCAGAAA TTTCACTGCA GATGCAGTTA 2820
CTTAGGGGAT TGTGGCAAAT TTGTCCACAA GCTTACTTTT CAATGGTTTC AGAACATTTA 2880
CTGGTTCTTG CCCTGAAAAC TGCTTATCAA AGCAACACTG TTGCTGCGTG TTCTCCATTT 2940
GTTTTGCAAT TCCTTCAAGG GAGAACACTG ACTTTGGGTG CGCTTAACTT ACAGTACTTT 3000
TTCGACCACC CAGAAAGCTT GTCATTGTTG AGGAGCATCC ACTTCCCAAT ACGAGGAAAT 3060
AAGACATCAC CCAGAGCACA TTTTTCAGTT CTGGAAACAT GTTTTGACAA ATCACAGGTG 3120
CCAACTATAG ATCAGGACTA TGCTTCTGCC TTTGAACCTA TGAATGAATG GGAGCGAAAT 3180
TTAGCTGAAA AAGAGGATAA TGTAAAGAGC TATATGGATA TGCAGCGCAG GGCATCACCA 3240
GACCTTAGTA CTGGCTATTG GAAACTTTCT CCAAAGCAGT ACAAGATTCC CTGTCTAGAA 3300
GTCGATGTGA ATGATATTGA TGTTGTAGGC CAGGATATGC TTGAGATTCT AATGACAGTT 3360
TTCTCAGCTT CACAGCGCAT CGAACTCCAT TTAAACCACA GCAGAGGCTT TATAGAAAGC 3420
ATCCGCCCAG CTCTTGAGCT GTCTAAGGCC TCTGTCACCA AGTGCTCCAT AAGCAAGTTG 3480
GAACTCAGCG CAGCCGAACA GGAACTGCTT CTCACCCTGC CTTCCCTGGA ATCTCTTGAA 3540
GTCTCAGGGA CAATCCAGTC ACAAGACCAA ATCTTTCCTA ATCTGGATAA GTTCCTGTGC 3600
CTGAAAGAAC TGTCTGTGGA TCTGGAGGGC AATATAAATG TTTTTTCAGT CATTCCTGAA 3660
GAATTTCCAA ACTTCCACCA TATGGAGAAA TTATTGATCC AAATTTCAGC TGAGTATGAT 3720
CCTTCCAAAC TAGTAAAATT AATTCAAAAT TCTCCAAACC TTCATGTTTT CCATCTGAAG 3780
TGTAACTTCT TTTCGGATTT TGGGTCTCTC ATGACTATGC TTGTTTCCTG TAAGAAACTC 3840
ACAGAAATTA AGTTTTCGGA TTCATTTTTT CAAGCCGTCC CATTTGTTGC CAGTTTGCCA 3900
AATTTTATTT CTCTGAAGAT ATTAAATCTT GAAGGCCAGC AATTTCCTGA TGAGGAAACA 3960
TCAGAAAAAT TTGCCTACAT TTTAGGTTCT CTTAGTAACC TGGAAGAATT GATCCTTCCT 4020
ACTGGGGATG GAATTTATCG AGTGGCCAAA CTGATCATCC AGCAGTGTCA GCAGCTTCAT 4080
TGTCTCCGAG TCCTCTCATT TTTCAAGACT TTGAATGATG ACAGCGTGGT GGAAATTGCC 4140
AAAGTAGCAA TCAGTGGAGG TTTCCAGAAA CTTGAGAACC TAAAGCTTTC AATCAATCAC 4200
AAGATTACAG AGGAAGGATA CAGAAATTTC TTTCAAGCAC TGGACAACAT GCCAAACTTG 4260
CAGGAGTTGG ACATCTCCAG GCATTTCACA GAGTGTATCA AAGCTCAGGC CACAACAGTC 4320
AAGTCTTTGA GTCAATGTGT GTTACGACTA CCAAGGCTCA TTAGACTGAA CATGTTAAGT 4380
TGGCTCTTGG ATGCAGATGA TATTGCATTG CTTAATGTCA TGAAAGAAAG ACATCCTCAA 4440
TCTAAGTACT TAACTATTCT CCAGAAATGG ATACTGCCGT TCTCTCCAAT CATTCAGAAA 4500
TAAAAGATTC AGCTAAAAAC TGCTGAATCA ATAATTTGTC TTGGGGCATA TTGAGGATGT 4560
AAAAAAAGTT GTTGATTAAT GCTAAAAACC AAATTATCCA AAATTATTTT ATTAAATATT 4620
GCATACAAAA GAAAATGTGT AAGGCTTGCT AAAAAACAAA ACAAAACAAA ACACAGTCCT 4680
GCATACTCAC CACCAAGCTC AAGAAATAAA TCATCACCAA TACCTTTGAG GTCCCTGAGT 4740
AATCCACCCC AGCTAAAGGC AAACCCTTCA ATCAAGTTTA TACAGCAAAC CCTCCATTGT 4800
CCATGGTCAA CAGGGAAGGG GTTGGGGACA GGTCTGCCAA TCTATCTAAA AGCCACAATA 4860
TGGAAGAAGT ATTCAATTTA TATAATAAAT GGCTAACTTA ACGGTTGAAT CACTTTCATA 4920
CATGGATGAA ACGGGTTTAA CACAGGATCC ACATGAATCT TCTGTGGGCC AAAATATGTT 4980
CCTTAATCCT TGTAGAACCT GTCTTCTATA TTGAACTAGC TTTGGTACAG TAGAGTTAAC 5040
TTACTTTCCA TTTATCCACT GCCAATATAA AGAGGAAACA GGGGTTAGGG AAAAATGACT 5100
TCATTCCAGA GGCTTCTCAG AGTTCAACAT ATGCTATAAT TTAGAATTTT CTTATGAATC 5160

CACTCTACTT GGGTAGAAAA TATTTTATCT CTAGTGATTG CATATTATTT CCATATCATA 5220
GTATTTCATA GTATTATATT TGATATGAGT GTCTATATCA ATGTCAGTGT CCAGAATTTC 5280
GTTCCTACCA GTTGAGTAGT TTTCTGAACG GCCAGAAGAC CATTCGAAAT TCATGATACT 5340
ACTATAAGTT GGTAAACAAC CATACTTTTA TCCTCATTTT TATTCTCACT AAGAAAAAAG 5400
TCAACTCCCC TCCCCTTGCC CAAGTATGAA ATATAGGGAC AGTATGTATG GTGTGGTCTC 5460
ATTTGTTTAG AAAACCACTT ATGACTGGGT GCGGTGGCTC ACACCTGTAA TCCCAGCACT 5520
TTGGGAGGCT GAGGCGGGCG AATCATTTGA GGTGAGGAGT TCGAGACCGG CCTGGCCAGC 5580
ATGGTGAAAC CCCATTTTTG CTAAAGGTAC AAAAATTAGC CAGGTGTGGT GGCACATGCC 5640
TGTGGTCCCA GCCACTGGGG CGGCTGAGAC GCAGGACTTG CTTGAACCCG GGAGGCAGAG 5700
GTTGCAGTGA GCCGAGATGG CGCCACTGCA TTCCAGCCTG GGCAACAGAG CAAGACCCTG 5760
TCTGTTTCAA AACAAAAAAC AAAACCACTT ATATTGCTAG CTACATTAAG AATTTCTGAA 5820
TATGTTACTG AGCTTGCTTG TGGTAACCAT TTATAATATC AGAAAGTATA TGTACACCAA 5880
AACATGTTGA ACATCCATGT TGTACAACTG AAATATAAAT AATTTTGTCA ATTATACCTA 5940
AATAAAACTG GAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAA 5984

配列番号：２　　トポロジー：直鎖状
配列の長さ：5366　　配列の種類：cDNA to mRNA
配列の型：核酸　　起源
鎖の数：二本鎖　　生物名：ヒト

配列

ACAAAAGGTC CTGTGCTCAC CTGGGACCCT TCTGGACGTT GCCCTGTGTT CCTCTTCGCC 60
TGCCTGTTCA TCTACGACGA ACCCCGGGTA TTGACCCCAG ACAACAATGC CACTTCATAT 120
TGGGGACTTC GTCTGGGATT CCAAGGTGCA TTCATTGCAA AGTTCCTTAA ATATTTTCTC 180
ACTGCTTCCT ACTAAAGGAC GGACAGAGCA TTTGTTCTTC AGCCACATAC TTTCCTTCCA 240
CTGGCCAGCA TTCTCCTCTA TTAGACTAGA ACTGTGGATA AACCTCAGAA AATGGCCACC 300
CAGCAGAAAG CCTCTGACGA GAGGATCTCC CAGTTTGATC ACAATTTGCT GCCAGAGCTG 360
TCTGCTCTTC TGGGCCTAGA TGCAGTTCAG TTGGCAAAGG AACTAGAAGA AGAGGAGCAG 420
AAGGAGCGAG CAAAAATGCA GAAAGGCTAC AACTCTCAAA TGCGCAGTGA AGCAAAAAGG 480
TTAAAGACTT TTGTGACTTA TGAGCCGTAC AGCTCATGGA TACCACAGGA GATGGCGGCC 540
GCTGGGTTTT ACTTCACTGG GGTAAAATCT GGGATTCAGT GCTTCTGCTG TAGCCTAATC 600
CTCTTTGGTG CCGGCCTCAC GAGACTCCCC ATAGAAGACC ACAAGAGGTT TCATCCAGAT 660
TGTGGGTTCC TTTTGAACAA GGATGTTGGT AACATTGCCA AGTACGACAT AAGGGTGAAG 720
AATCTGAAGA GCAGGCTGAG AGGAGGTAAA ATGAGGTACC AAGAAGAGGA GGCTAGACTT 780
GCATCCTTCA GGAACTGGCC ATTTTATGTC CAAGGGATAT CCCCTTGTGT GCTCTCAGAG 840
GCTGGCTTTG TCTTTACAGG TAAACAGGAC ACGGTACAGT GTTTTTCCTG TGGTGGATGT 900
TTAGGAAATT GGGAAGAAGG AGATGATCCT TGGAAGGAAC ATGCCAAATG GTTCCCCAAA 960
TGTGAATTTC TTCGGAGTAA GAAATCCTCA GAGGAAATTA CCCAGTATAT TCAAAGCTAC 1020
AAGGGATTTG TTGACATAAC GGGAGAACAT TTTGTGAATT CCTGGGTCCA GAGAGAATTA 1080
CCTATGGCAT CAGCTTATTG CAATGACAGC ATCTTTGCTT ACGAAGAACT ACGGCTGGAC 1140
TCTTTTAAGG ACTGGCCCCG GGAATCAGCT GTGGGAGTTG CAGCACTGGC CAAAGCAGGT 1200
CTTTTCTACA CAGGTATAAA GGACATCGTC CAGTGCTTTT CCTGTGGAGG GTGTTTAGAG 1260
AAATGGCAGG AAGGTGATGA CCCATTAGAC GATCACACCA GATGTTTTCC CAATTGTCCA 1320
TTTCTCCAAA ATATGAAGTC CTCTGCGGAA GTGACTCCAG ACCTTCAGAG CCGTGGTGAA 1380
CTTTGTGAAT TACTGGAAAC CACAAGTGAA AGCAATCTTG AAGATTCAAT AGCAGTTGGT 1440
CCTATAGTGC CAGAAATGGC ACAGGGTGAA GCCCAGTGGT TTCAAGAGGC AAAGAATCTG 1500
AATGAGCAGC TGAGAGCAGC TTATACCAGC GCCAGTTTCC GCCACATGTC TTTGCTTGAT 1560
ATCTCTTCCG ATCTGGCCAC GGACCACTTG CTGGGCTGTG ATCTGTCTAT TGCTTCAAAA 1620
CACATCAGCA AACCTGTGCA AGAACCTCTG GTGCTGCCTG AGGTCTTTGG CAACTTGAAC 1680
TCTGTCATGT GTGTGGAGGG TGAAGCTGGA AGTGGAAAGA CGGTCCTCCT GAAGAAAATA 1740
GCTTTTCTGT GGGCATCTGG ATGCTGTCCC CTGTTAAACA GGTTCCAGCT GGTTTTCTAC 1800
CTCTCCCTTA GTTCCACCAG ACCAGACGAG GGGCTGGCCA GTATCATCTG TGACCAGCTC 1860

CTAGAGAAAG AAGGATCTGT TACTGAAATG TGCATGAGGA ACATTATCCA GCAGTTAAAG 1920
AATCAGGTCT TATTCCTTTT AGATGACTAC AAAGAAATAT GTTCAATCCC TCAAGTCATA 1980
GGAAAACTGA TTCAAAAAAA CCACTTATCC CGGACCTGCC TATTGATTGC TGTCCGTACA 2040
AACAGGGCCA GGGACATCCG CCGATACCTA GAGACCATTC TAGAGATCAA AGCATTTCCC 2100
TTTTATAATA CTGTCTGTAT ATTACGGAAG CTCTTTTCAC ATAATATGAC TCGTCTGCGA 2160
AAGTTTATGG TTTACTTTGG AAAGAACCAA AGTTTGCAGA AGATACAGAA AACTCCTCTC 2220
TTTGTGGCGG CGATCTGTGC TCATTGGTTT CAGTATCCTT TTGACCCATC CTTTGATGAT 2280
GTGGCTGTTT TCAAGTCCTA TATGGAACGC CTTTCCTTAA GGAACAAAGC GACAGCTGAA 2340
ATTCTCAAAG CAACTGTGTC CTCCTGTGGT GAGCTGGCCT TGAAAGGGTT TTTTTCATGT 2400
TGCTTTGAGT TTAATGATGA TGATCTCGCA GAAGCAGGGG TTGATGAAGA TGAAGATCTA 2460
ACCATGTGCT TGATGAGCAA ATTTACAGCC CAGAGACTAA GACCATTCTA CCGGTTTTTA 2520
AGTCCTGCCT TCCAAGAATT TCTTGCGGGG ATGAGGCTGA TTGAACTCCT GGATTCAGAT 2580
AGGCAGGAAC ATCAAGATTT GGGACTGTAT CATTTGAAAC AAATCAACTC ACCCATGATG 2640
ACTGTAAGCG CCTACAACAA TTTTTTGAAC TATGTCTCCA GCCTCCCTTC AACAAAAGCA 2700
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CTTAGGGGAT TGTGGCAAAT TTGTCCACAA GCTTACTTTT CAATGGTTTC AGAACATTTA 2880
CTGGTTCTTG CCCTGAAAAC TGCTTATCAA AGCAACACTG TTGCTGCGTG TTCTCCATTT 2940
GTTTTGCAAT TCCTTCAAGG GAGAACACTG ACTTTGGGTG CGCTTAACTT ACAGTACTTT 3000
TTCGACCACC CAGAAAGCTT GTCATTGTTG AGGAGCATCC ACTTCCCAAT ACGAGGAAAT 3060
AAGACATCAC CCAGAGCACA TTTTTCAGTT CTGGAAACAT GTTTTGACAA ATCACAGGTG 3120
CCAACTATAG ATCAGGACTA TGCTTCTGCC TTTGAACCTA TGAATGAATG GGAGCGAAAT 3180
TTAGCTGAAA AAGAGGATAA TGTAAAGAGC TATATGGATA TGCAGCGCAG GGCATCACCA 3240
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GTCGATGTGA ATGATATTGA TGTTGTAGGC CAGGATATGC TTGAGATTCT AATGACAGTT 3360
TTCTCAGCTT CACAGCGCAT CGAACTCCAT TTAAACCACA GCAGAGGCTT TATAGAAAGC 3420
ATCCGCCCAG CTCTTGAGCT GTCTAAGGCC TCTGTCACCA AGTGCTCCAT AAGCAAGTTG 3480
GAACTCAGCG CAGCCGAACA GGAACTGCTT CTCACCCTGC CTTCCCTGGA ATCTCTTGAA 3540
GTCTCAGGGA CAATCCAGTC ACAAGACCAA ATCTTTCCTA ATCTGGATAA GTTCCTGTGC 3600
CTGAAAGAAC TGTCTGTGGA TCTGGAGGGC AATATAAATG TTTTTTCAGT CATTCCTGAA 3660
GAATTTCCAA ACTTCCACCA TATGGAGAAA TTATTGATCC AAATTTCAGC TGAGTATGAT 3720
CCTTCCAAAC TAGTAAAATT AATTCAAAAT TCTCCAAACC TTCATGTTTT CCATCTGAAG 3780
TGTAACTTCT TTTCGGATTT TGGGTCTCTC ATGACTATGC TTGTTTCCTG TAAGAAACTC 3840
ACAGAAATTA AGTTTTCGGA TTCATTTTTT CAAGCCGTCC CATTTGTTGC CAGTTTGCCA 3900
AATTTTATTT CTCTGAAGAT ATTAAATCTT GAAGGCCAGC AATTTCCTGA TGAGGAAACA 3960
TCAGAAAAAT TTGCCTACAT TTTAGGTTCT CTTAGTAACC TGGAAGAATT GATCCTTCCT 4020
ACTGGGGATG GAATTTATCG AGTGGCCAAA CTGATCATCC AGCAGTGTCA GCAGCTTCAT 4080
TGTCTCCGAG TCCTCTCATT TTTCAAGACT TTGAATGATG ACAGCGTGGT GGAAATTGGT 4140
GAGCTAGTGT TTCAGCTTGC ATGGAAGCCA GTGGTATAGC CAAGCTTTCT GCTGCAACAT 4200
GTCTATGTAA ACATTTGCCC CTCTAGAAAT TTTCAACCCG CTTCCTCATT TTCACTATCA 4260
TACTGTTCCT TCTAGTGTCC TTCTGTGGAT TTAGGCGCAT TCTGGTCAGA TTTGGAAGTA 4320
CAAAAAGGTC TCCCATTTGT GGATATACAA GCCCTCAAAT CTGCGTTCTT GCCACCTGGT 4380
GTTTTAGACA CCTGGCCACA TACTCTCCTA AGTACTCCTT TTTAAAACTG AAGATGAATA 4440
TACACACAGA AAAGTACAAA AATCATGTGT ACTGCTCACT GAATTTTATT TTCTTATTTT 4500
CTTCTTTTTT TTTTTTTTGA GACAGAGTTT CGCTCGTGTT GCCCAGGCTG GAGTACAATG 4560
GCACGATCTC GGGTCACTGC AAACTCTGCC TCCTGGGTTC AAGCGATTCT CCTGCCTCAG 4620
CCTCCCAAGT AGCTAGGATT ACAGGTGAAC GCCACCACAC CTGGCTAATT TTGTATTTTT 4680
AGTAAACACA GGGTTTCACC ATGTTGGCCA GGCTAGTCTC GAACTCCTGA CCTCAAGTGA 4740
GCCACAGTGC CTGGCCTGAG GAACTGAGAT TTCTGTCGAG ACCTGAAGGG AGAATGGCCC 4800
AGGCATAGTT GGTAGAGGAG GAATTGAGAC ATCATTTCAA ACAGAGGTAA TCACTTGTGT 4860

19 20

CATAGCCTGG AGTTAAAGAG AACCAGATAT ATTTGAAGAA CTTGGGGGAA AAAAAGGAAT 4920
GTCTGGAGCA AGAGGCAGGA GTGAGTTGTG AGAAGAAGAC TGGAGAGGAA AGTAAAAGCC 4980
CAATTGGAGA GGCTTTGTCG GGTGTGTTAC AAGGGCTGGA TCTCATTTTC TTACTGCTCA 5040
GCACTGTTAT TTTACGTTAT TTAAAACAGC TGGGAGCGGT GGCTCAAGCT TGTAATCCCA 5100
GCACTTTGGG AGGCCGAGGC GGATGGATCA CGAGGTCAGG AGATCGAGAC CATCCTGGCT 5160
AACATGGTGA AACCCCGTCT CTACTAAAAA TACAAAAAAT TAGCCAGGCG TGATGGCGGG 5220
CACCTGTAGT CCCAGCTACT CGGGAGGCTG AGGCAGGAGA ATGGTGTGAA CCCGGGAGGT 5280
GGAGCTTGAA GTGAGCCAAG ATCATGCCAC TGCACTCCAG CCTGGGCAAC AGAACGAGAC 5340
TCCGTCTCAA AAAAAAAAAA CAAAAA 5366

配列番号：３ 10 トポロジー：直鎖状
配列の長さ：1404 配列の種類：タンパク質
配列の型：アミノ酸

配列

Met Ala Thr Gln Gln Lys Ala Ser Asp Glu Arg Ile Ser Gln Phe Asp
1 5 10 15
His Asn Leu Leu Pro Glu Leu Ser Ala Leu Leu Gly Leu Asp Ala Val
20 25 30
Gln Leu Ala Lys Glu Leu Glu Glu Glu Glu Gln Lys Glu Arg Ala Lys
35 40 45
Met Gln Lys Gly Tyr Asn Ser Gln Met Arg Ser Glu Ala Lys Arg Leu
50 55 60
Lys Thr Phe Val Thr Tyr Glu Pro Tyr Ser Ser Trp Ile Pro Gln Glu
65 70 75 80
Met Ala Ala Ala Gly Phe Tyr Phe Thr Gly Val Lys Ser Gly Ile Gln
85 90 95
Cys Phe Cys Cys Ser Leu Ile Leu Phe Gly Ala Gly Leu Thr Arg Leu
100 105 110
Pro Ile Glu Asp His Lys Arg Phe His Pro Asp Cys Gly Phe Leu Leu
115 120 125
Asn Lys Asp Val Gly Asn Ile Ala Lys Tyr Asp Ile Arg Val Lys Asn
130 135 140
Leu Lys Ser Arg Leu Arg Gly Gly Lys Met Arg Tyr Gln Glu Glu Glu
145 150 155 160
Ala Arg Leu Ala Ser Phe Arg Asn Trp Pro Phe Tyr Val Gln Gly Ile
165 170 175
Ser Pro Cys Val Leu Ser Glu Ala Gly Phe Val Phe Thr Gly Lys Gln
180 185 190
Asp Thr Val Gln Cys Phe Ser Cys Gly Gly Cys Leu Gly Asn Trp Glu
195 200 205
Glu Gly Asp Asp Pro Trp Lys Glu His Ala Lys Trp Phe Pro Lys Cys
210 215 220
Glu Phe Leu Arg Ser Lys Lys Ser Ser Glu Glu Ile Thr Gln Tyr Ile
225 230 235 240
Gln Ser Tyr Lys Gly Phe Val Asp Ile Thr Gly Glu His Phe Val Asn
245 250 255
Ser Trp Val Gln Arg Glu Leu Pro Met Ala Ser Ala Tyr Cys Asn Asp
260 265 270
Ser Ile Phe Ala Tyr Glu Glu Leu Arg Leu Asp Ser Phe Lys Asp Trp
275 280 285
Pro Arg Glu Ser Ala Val Gly Val Ala Ala Leu Ala Lys Ala Gly Leu

290 295 300

Phe Tyr Thr Gly Ile Lys Asp Ile Val Gln Cys Phe Ser Cys Gly Gly
305 310 315 320
Cys Leu Glu Lys Trp Gln Glu Gly Asp Asp Pro Leu Asp Asp His Thr
325 330 335
Arg Cys Phe Pro Asn Cys Pro Phe Leu Gln Asn Met Lys Ser Ser Ala
340 345 350
Glu Val Thr Pro Asp Leu Gln Ser Arg Gly Glu Leu Cys Glu Leu Leu
355 360 365
Glu Thr Thr Ser Glu Ser Asn Leu Glu Asp Ser Ile Ala Val Gly Pro
370 375 380
Ile Val Pro Glu Met Ala Gln Gly Glu Ala Gln Trp Phe Gln Glu Ala
385 390 395 400
Lys Asn Leu Asn Glu Gln Leu Arg Ala Ala Tyr Thr Ser Ala Ser Phe
405 410 415
Arg His Met Ser Leu Leu Asp Ile Ser Ser Asp Leu Ala Thr Asp His
420 425 430
Leu Leu Gly Cys Asp Leu Ser Ile Ala Ser Lys His Ile Ser Lys Pro
435 440 445
Val Gln Glu Pro Leu Val Leu Pro Glu Val Phe Gly Asn Leu Asn Ser
450 455 460
Val Met Cys Val Glu Gly Glu Ala Gly Ser Gly Lys Thr Val Leu Leu
465 470 475 480
Lys Lys Ile Ala Phe Leu Trp Ala Ser Gly Cys Cys Pro Leu Leu Asn
485 490 495
Arg Phe Gln Leu Val Phe Tyr Leu Ser Leu Ser Ser Thr Arg Pro Asp
500 505 510
Glu Gly Leu Ala Ser Ile Ile Cys Asp Gln Leu Leu Glu Lys Glu Gly
515 520 525
Ser Val Thr Glu Met Cys Met Arg Asn Ile Ile Gln Gln Leu Lys Asn
530 535 540
Gln Val Leu Phe Leu Leu Asp Asp Tyr Lys Glu Ile Cys Ser Ile Pro
545 550 555 560
Gln Val Ile Gly Lys Leu Ile Gln Lys Asn His Leu Ser Arg Thr Cys
565 570 575
Leu Leu Ile Ala Val Arg Thr Asn Arg Ala Arg Asp Ile Arg Arg Tyr
580 585 590
Leu Glu Thr Ile Leu Glu Ile Lys Ala Phe Pro Phe Tyr Asn Thr Val
595 600 605
Cys Ile Leu Arg Lys Leu Phe Ser His Asn Met Thr Arg Leu Arg Lys
610 615 620
Phe Met Val Tyr Phe Gly Lys Asn Gln Ser Leu Gln Lys Ile Gln Lys
625 630 635 640
Thr Pro Leu Phe Val Ala Ala Ile Cys Ala His Trp Phe Gln Tyr Pro
645 650 655
Phe Asp Pro Ser Phe Asp Asp Val Ala Val Phe Lys Ser Tyr Met Glu
660 665 670
Arg Leu Ser Leu Arg Asn Lys Ala Thr Ala Glu Ile Leu Lys Ala Thr
675 680 685
Val Ser Ser Cys Gly Glu Leu Ala Leu Lys Gly Phe Phe Ser Cys Cys

690 695 700

Phe Glu Phe Asn Asp Asp Asp Leu Ala Glu Ala Gly Val Asp Glu Asp
705 710 715 720
Glu Asp Leu Thr Met Cys Leu Met Ser Lys Phe Thr Ala Gln Arg Leu
725 730 735
Arg Pro Phe Tyr Arg Phe Leu Ser Pro Ala Phe Gln Glu Phe Leu Ala
740 745 750
Gly Met Arg Leu Ile Glu Leu Leu Asp Ser Asp Arg Gln Glu His Gln
755 760 765
Asp Leu Gly Leu Tyr His Leu Lys Gln Ile Asn Ser Pro Met Met Thr
770 775 780
Val Ser Ala Tyr Asn Asn Phe Leu Asn Tyr Val Ser Ser Leu Pro Ser
785 790 795 800
Thr Lys Ala Gly Pro Lys Ile Val Ser His Leu Leu His Leu Val Asp
805 810 815
Asn Lys Glu Ser Leu Glu Asn Ile Ser Glu Asn Asp Asp Tyr Leu Lys
820 825 830
His Gln Pro Glu Ile Ser Leu Gln Met Gln Leu Leu Arg Gly Leu Trp
835 840 845
Gln Ile Cys Pro Gln Ala Tyr Phe Ser Met Val Ser Glu His Leu Leu
850 855 860
Val Leu Ala Leu Lys Thr Ala Tyr Gln Ser Asn Thr Val Ala Ala Cys
865 870 875 880
Ser Pro Phe Val Leu Gln Phe Leu Gln Gly Arg Thr Leu Thr Leu Gly
885 890 895
Ala Leu Asn Leu Gln Tyr Phe Phe Asp His Pro Glu Ser Leu Ser Leu
900 905 910
Leu Arg Ser Ile His Phe Pro Ile Arg Gly Asn Lys Thr Ser Pro Arg
915 920 925
Ala His Phe Ser Val Leu Glu Thr Cys Phe Asp Lys Ser Gln Val Pro
930 935 940
Thr Ile Asp Gln Asp Tyr Ala Ser Ala Phe Glu Pro Met Asn Glu Trp
945 950 955 960
Glu Arg Asn Leu Ala Glu Lys Glu Asp Asn Val Lys Ser Tyr Met Asp
965 970 975
Met Gln Arg Arg Ala Ser Pro Asp Leu Ser Thr Gly Tyr Trp Lys Leu
980 985 990
Ser Pro Lys Gln Tyr Lys Ile Pro Cys Leu Glu Val Asp Val Asn Asp
995 1000 1005
Ile Asp Val Val Gly Gln Asp Met Leu Glu Ile Leu Met Thr Val Phe
1010 1015 1020
Ser Ala Ser Gln Arg Ile Glu Leu His Leu Asn His Ser Arg Gly Phe
1025 1030 1035 1040
Ile Glu Ser Ile Arg Pro Ala Leu Glu Leu Ser Lys Ala Ser Val Thr
1045 1050 1055
Lys Cys Ser Ile Ser Lys Leu Glu Leu Ser Ala Ala Glu Gln Glu Leu
1060 1065 1070
Leu Leu Thr Leu Pro Ser Leu Glu Ser Leu Glu Val Ser Gly Thr Ile
1075 1080 1085
Gln Ser Gln Asp Gln Ile Phe Pro Asn Leu Asp Lys Phe Leu Cys Leu

1090 1095 1100
Lys Glu Leu Ser Val Asp Leu Glu Gly Asn Ile Asn Val Phe Ser Val
1105 1110 1115 1120
Ile Pro Glu Glu Phe Pro Asn Phe His His Met Glu Lys Leu Leu Ile
1125 1130 1135
Gln Ile Ser Ala Glu Tyr Asp Pro Ser Lys Leu Val Lys Leu Ile Gln
1140 1145 1150
Asn Ser Pro Asn Leu His Val Phe His Leu Lys Cys Asn Phe Phe Ser
1155 1160 1165
Asp Phe Gly Ser Leu Met Thr Met Leu Val Ser Cys Lys Lys Leu Thr
1170 1175 1180
Glu Ile Lys Phe Ser Asp Ser Phe Phe Gln Ala Val Pro Phe Val Ala
1185 1190 1195 1200
Ser Leu Pro Asn Phe Ile Ser Leu Lys Ile Leu Asn Leu Glu Gly Gln
1205 1210 1215
Gln Phe Pro Asp Glu Glu Thr Ser Glu Lys Phe Ala Tyr Ile Leu Gly
1220 1225 1230
Ser Leu Ser Asn Leu Glu Glu Leu Ile Leu Pro Thr Gly Asp Gly Ile
1235 1240 1245
Tyr Arg Val Ala Lys Leu Ile Ile Gln Gln Cys Gln Gln Leu His Cys
1250 1255 1260
Leu Arg Val Leu Ser Phe Phe Lys Thr Leu Asn Asp Asp Ser Val Val
1265 1270 1275 1280
Glu Ile Ala Lys Val Ala Ile Ser Gly Gly Phe Gln Lys Leu Glu Asn
1285 1290 1295
Leu Lys Leu Ser Ile Asn His Lys Ile Thr Glu Glu Gly Tyr Arg Asn
1300 1305 1310
Phe Phe Gln Ala Leu Asp Asn Met Pro Asn Leu Gln Glu Leu Asp Ile
1315 1320 1325
Ser Arg His Phe Thr Glu Cys Ile Lys Ala Gln Ala Thr Thr Val Lys
1330 1335 1340
Ser Leu Ser Gln Cys Val Leu Arg Leu Pro Arg Leu Ile Arg Leu Asn
1345 1350 1355 1360
Met Leu Ser Trp Leu Leu Asp Ala Asp Asp Ile Ala Leu Leu Asn Val
1365 1370 1375
Met Lys Glu Arg His Pro Gln Ser Lys Tyr Leu Thr Ile Leu Gln Lys
1380 1385 1390
Trp Ile Leu Pro Phe Ser Pro Ile Ile Gln Lys
1395 1400 1403

配列番号：４ トポロジー：直鎖状
配列の長さ：1295 配列の種類：タンパク質
配列の型：アミノ酸

配列

Met Ala Thr Gln Gln Lys Ala Ser Asp Glu Arg Ile Ser Gln Phe Asp
1 5 10 15
His Asn Leu Leu Pro Glu Leu Ser Ala Leu Leu Gly Leu Asp Ala Val
20 25 30
Gln Leu Ala Lys Glu Leu Glu Glu Glu Glu Gln Lys Glu Arg Ala Lys
35 40 45
Met Gln Lys Gly Tyr Asn Ser Gln Met Arg Ser Glu Ala Lys Arg Leu

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     50                  55                  60
Lys Thr Phe Val Thr Tyr Glu Pro Tyr Ser Ser Trp Ile Pro Gln Glu
 65                  70                  75                  80
Met Ala Ala Ala Gly Phe Tyr Phe Thr Gly Val Lys Ser Gly Ile Gln
                 85                  90                  95
Cys Phe Cys Cys Ser Leu Ile Leu Phe Gly Ala Gly Leu Thr Arg Leu
            100                 105                 110
Pro Ile Glu Asp His Lys Arg Phe His Pro Asp Cys Gly Phe Leu Leu
        115                 120                 125
Asn Lys Asp Val Gly Asn Ile Ala Lys Tyr Asp Ile Arg Val Lys Asn
    130                 135                 140
Leu Lys Ser Arg Leu Arg Gly Gly Lys Met Arg Tyr Gln Glu Glu Glu
145                 150                 155                 160
Ala Arg Leu Ala Ser Phe Arg Asn Trp Pro Phe Tyr Val Gln Gly Ile
                165                 170                 175
Ser Pro Cys Val Leu Ser Glu Ala Gly Phe Val Phe Thr Gly Lys Gln
            180                 185                 190
Asp Thr Val Gln Cys Phe Ser Cys Gly Gly Cys Leu Gly Asn Trp Glu
        195                 200                 205
Glu Gly Asp Asp Pro Trp Lys Glu His Ala Lys Trp Phe Pro Lys Cys
    210                 215                 220
Glu Phe Leu Arg Ser Lys Lys Ser Ser Glu Glu Ile Thr Gln Tyr Ile
225                 230                 235                 240
Gln Ser Tyr Lys Gly Phe Val Asp Ile Thr Gly Glu His Phe Val Asn
                245                 250                 255
Ser Trp Val Gln Arg Glu Leu Pro Met Ala Ser Ala Tyr Cys Asn Asp
            260                 265                 270
Ser Ile Phe Ala Tyr Glu Glu Leu Arg Leu Asp Ser Phe Lys Asp Trp
        275                 280                 285
Pro Arg Glu Ser Ala Val Gly Val Ala Ala Leu Ala Lys Ala Gly Leu
    290                 295                 300
Phe Tyr Thr Gly Ile Lys Asp Ile Val Gln Cys Phe Ser Cys Gly Gly
305                 310                 315                 320
Cys Leu Glu Lys Trp Gln Glu Gly Asp Asp Pro Leu Asp Asp His Thr
                325                 330                 335
Arg Cys Phe Pro Asn Cys Pro Phe Leu Gln Asn Met Lys Ser Ser Ala
            340                 345                 350
Glu Val Thr Pro Asp Leu Gln Ser Arg Gly Glu Leu Cys Glu Leu Leu
        355                 360                 365
Glu Thr Thr Ser Glu Ser Asn Leu Glu Asp Ser Ile Ala Val Gly Pro
    370                 375                 380
Ile Val Pro Glu Met Ala Gln Gly Glu Ala Gln Trp Phe Gln Glu Ala
385                 390                 395                 400
Lys Asn Leu Asn Glu Gln Leu Arg Ala Ala Tyr Thr Ser Ala Ser Phe
                405                 410                 415
Arg His Met Ser Leu Leu Asp Ile Ser Ser Asp Leu Ala Thr Asp His
            420                 425                 430
Leu Leu Gly Cys Asp Leu Ser Ile Ala Ser Lys His Ile Ser Lys Pro
        435                 440                 445
Val Gln Glu Pro Leu Val Leu Pro Glu Val Phe Gly Asn Leu Asn Ser
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    450                 455                 460
Val Met Cys Val Glu Gly Glu Ala Gly Ser Gly Lys Thr Val Leu Leu
465                 470                 475                 480
Lys Lys Ile Ala Phe Leu Trp Ala Ser Gly Cys Cys Pro Leu Leu Asn
                485                 490                 495
Arg Phe Gln Leu Val Phe Tyr Leu Ser Leu Ser Ser Thr Arg Pro Asp
            500                 505                 510
Glu Gly Leu Ala Ser Ile Ile Cys Asp Gln Leu Leu Glu Lys Glu Gly
        515                 520                 525
Ser Val Thr Glu Met Cys Met Arg Asn Ile Ile Gln Gln Leu Lys Asn
    530                 535                 540
Gln Val Leu Phe Leu Leu Asp Asp Tyr Lys Glu Ile Cys Ser Ile Pro
545                 550                 555                 560
Gln Val Ile Gly Lys Leu Ile Gln Lys Asn His Leu Ser Arg Thr Cys
                565                 570                 575
Leu Leu Ile Ala Val Arg Thr Asn Arg Ala Arg Asp Ile Arg Arg Tyr
            580                 585                 590
Leu Glu Thr Ile Leu Glu Ile Lys Ala Phe Pro Phe Tyr Asn Thr Val
        595                 600                 605
Cys Ile Leu Arg Lys Leu Phe Ser His Asn Met Thr Arg Leu Arg Lys
    610                 615                 620
Phe Met Val Tyr Phe Gly Lys Asn Gln Ser Leu Gln Lys Ile Gln Lys
625                 630                 635                 640
Thr Pro Leu Phe Val Ala Ala Ile Cys Ala His Trp Phe Gln Tyr Pro
                645                 650                 655
Phe Asp Pro Ser Phe Asp Asp Val Ala Val Phe Lys Ser Tyr Met Glu
            660                 665                 670
Arg Leu Ser Leu Arg Asn Lys Ala Thr Ala Glu Ile Leu Lys Ala Thr
        675                 680                 685
Val Ser Ser Cys Gly Glu Leu Ala Leu Lys Gly Phe Phe Ser Cys Cys
    690                 695                 700
Phe Glu Phe Asn Asp Asp Asp Leu Ala Glu Ala Gly Val Asp Glu Asp
705                 710                 715                 720
Glu Asp Leu Thr Met Cys Leu Met Ser Lys Phe Thr Ala Gln Arg Leu
                725                 730                 735
Arg Pro Phe Tyr Arg Phe Leu Ser Pro Ala Phe Gln Glu Phe Leu Ala
            740                 745                 750
Gly Met Arg Leu Ile Glu Leu Leu Asp Ser Asp Arg Gln Glu His Gln
        755                 760                 765
Asp Leu Gly Leu Tyr His Leu Lys Gln Ile Asn Ser Pro Met Met Thr
    770                 775                 780
Val Ser Ala Tyr Asn Asn Phe Leu Asn Tyr Val Ser Ser Leu Pro Ser
785                 790                 795                 800
Thr Lys Ala Gly Pro Lys Ile Val Ser His Leu Leu His Leu Val Asp
                805                 810                 815
Asn Lys Glu Ser Leu Glu Asn Ile Ser Glu Asn Asp Asp Tyr Leu Lys
            820                 825                 830
His Gln Pro Glu Ile Ser Leu Gln Met Gln Leu Leu Arg Gly Leu Trp
        835                 840                 845
Gln Ile Cys Pro Gln Ala Tyr Phe Ser Met Val Ser Glu His Leu Leu
```

850 855 860
Val Leu Ala Leu Lys Thr Ala Tyr Gln Ser Asn Thr Val Ala Ala Cys
865 870 875 880
Ser Pro Phe Val Leu Gln Phe Leu Gln Gly Arg Thr Leu Thr Leu Gly
885 890 895
Ala Leu Asn Leu Gln Tyr Phe Phe Asp His Pro Glu Ser Leu Ser Leu
900 905 910
Leu Arg Ser Ile His Phe Pro Ile Arg Gly Asn Lys Thr Ser Pro Arg
915 920 925
Ala His Phe Ser Val Leu Glu Thr Cys Phe Asp Lys Ser Gln Val Pro
930 935 940
Thr Ile Asp Gln Asp Tyr Ala Ser Ala Phe Glu Pro Met Asn Glu Trp
945 950 955 960
Glu Arg Asn Leu Ala Glu Lys Glu Asp Asn Val Lys Ser Tyr Met Asp
965 970 975
Met Gln Arg Arg Ala Ser Pro Asp Leu Ser Thr Gly Tyr Trp Lys Leu
980 985 990
Ser Pro Lys Gln Tyr Lys Ile Pro Cys Leu Glu Val Asp Val Asn Asp
995 1000 1005
Ile Asp Val Val Gly Gln Asp Met Leu Glu Ile Leu Met Thr Val Phe
1010 1015 1020
Ser Ala Ser Gln Arg Ile Glu Leu His Leu Asn His Ser Arg Gly Phe
1025 1030 1035 1040
Ile Glu Ser Ile Arg Pro Ala Leu Glu Leu Ser Lys Ala Ser Val Thr
1045 1050 1055
Lys Cys Ser Ile Ser Lys Leu Glu Leu Ser Ala Ala Glu Gln Glu Leu
1060 1065 1070
Leu Leu Thr Leu Pro Ser Leu Glu Ser Leu Glu Val Ser Gly Thr Ile
1075 1080 1085
Gln Ser Gln Asp Gln Ile Phe Pro Asn Leu Asp Lys Phe Leu Cys Leu
1090 1095 1100
Lys Glu Leu Ser Val Asp Leu Glu Gly Asn Ile Asn Val Phe Ser Val
1105 1110 1115 1120
Ile Pro Glu Glu Phe Pro Asn Phe His His Met Glu Lys Leu Leu Ile
1125 1130 1135
Gln Ile Ser Ala Glu Tyr Asp Pro Ser Lys Leu Val Lys Leu Ile Gln
1140 1145 1150
Asn Ser Pro Asn Leu His Val Phe His Leu Lys Cys Asn Phe Phe Ser
1155 1160 1165
Asp Phe Gly Ser Leu Met Thr Met Leu Val Ser Cys Lys Lys Leu Thr
1170 1175 1180
Glu Ile Lys Phe Ser Asp Ser Phe Phe Gln Ala Val Pro Phe Val Ala
1185 1190 1195 1200
Ser Leu Pro Asn Phe Ile Ser Leu Lys Ile Leu Asn Leu Glu Gly Gln
1205 1210 1215
Gln Phe Pro Asp Glu Glu Thr Ser Glu Lys Phe Ala Tyr Ile Leu Gly
1220 1225 1230
Ser Leu Ser Asn Leu Glu Glu Leu Ile Leu Pro Thr Gly Asp Gly Ile
1235 1240 1245
Tyr Arg Val Ala Lys Leu Ile Ile Gln Gln Cys Gln Gln Leu His Cys

33　　34

1250　　1255　　1260
Leu Arg Val Leu Ser Phe Phe Lys Thr Leu Asn Asp Asp Ser Val Val
1265　　1270　　1275　　1280
Glu Ile Gly Glu Leu Val Phe Gln Leu Ala Trp Lys Pro Val Val
1285　　1290　　1295

【図面の簡単な説明】

【図１】卵巣内における卵胞の発達過程を示した模式図である。

【図２】ハイブリダイゼーション用プローブの遺伝子位置を示した模式図である。

【図３】卵巣におけるＮＡＩＰ遺伝子の発現を調べたin situ hybridization の結果であり、(A) はセンスリボプローブを用いた場合、(B) はアンチセンスリボプローブを用いた場合を示す。

【図４】マウスＮＡＩＰ遺伝子のノーザンブロット分析の結果である。(A) はマウス各組織での遺伝子発現を示し、各レーンは、１：精巣、２：腎臓、３：骨格筋、４：肝臓、５：肺、６：脾臓、７：脳、８：心臓である。(B) はマウスの発達過程における卵巣内での遺伝子発現を示し、各レーンは、１：２日令、２：３週令、３：12週令、４：分娩３日目、５：18週令である。

【図５】卵巣内におけるＮＡＩＰ遺伝子の発現が顆粒膜細胞に局在していることを示したin situ hybridization （上段、中断）およびＴＵＮＥＬ法（下段）の結果である。

【図１】

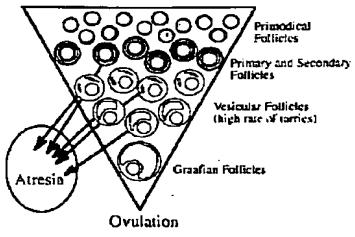
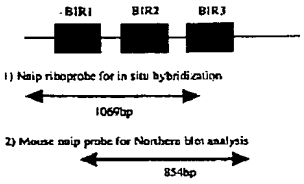
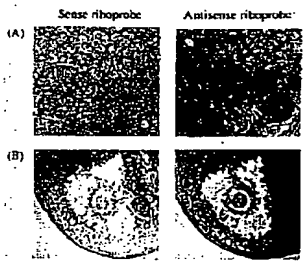


Fig. 1. Follicular development in the mouse ovary

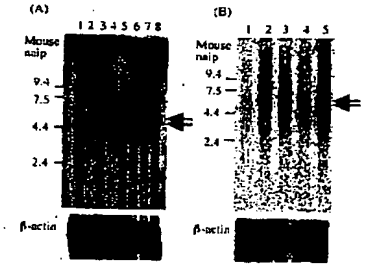
【図２】



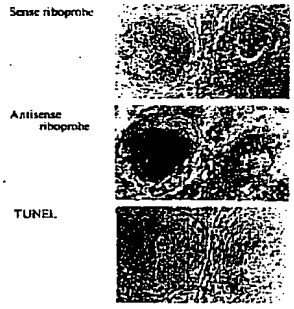
【図３】



【図４】



【図５】



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